INVESTIGATION OF THE BIOAVAILABILITY OF SEDIMENT-ASSOCIATED HYDROPHOBIC ORGANIC CONTAMINANTS VIA LABORATORY BIOASSAYS

A Dissertation

Presented to

the Graduate School of

Clemson University

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Environmental Toxicology

---

by

Gail Ann Harkey

December 1993

December 9. 1993

To the Graduate School:

This dissertation entitled "Investigation of the Bioavailability of Sediment-Associated Hydrophobic Organic Contaminants via Laboratory Bioassays" and written by Gail Ann Harkey is presented to the Graduate School of Clemson University. I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy with a major in Environmental Toxicology.

Dissertation Advisor, Clemson University

Dissertation Advisor, Ann Arbor, MI.

We have reviewed this dissertation
and recommend its acceptance:

Accepted for the Graduate School:

ABSTRACT

Bioassays have frequently been used as tools to simulate exposure of benthos to sediment-associated contaminants in hazard assessments. Due to the problems involved with diluting and estimating bioavailability in whole sediment bioassays, aqueous fractions, such as porewater and elutriates, have been substituted for whole sediment exposures. The objective of this research was to compare and evaluate the bioavailability of neutral hydrophobic contaminants in whole sediment and in aqueous extracts of whole sediment (elutriate and porewater) in simulataneous bioassays, using three representative indicator species. Data showed that aqueous extracts of whole sediment, in most cases, underexposed organisms compared to whole sediment, even after adjusting accumulation to the fraction of organic carbon contained in the test media. Bioaccumulation relationships among the media varied with sampling time and with the species used.

Subsequent experiments were conducted to identify the factors responsible for the observed bioaccumulation differences. Partitioning studies showed differential partitioning of the contaminants in porewater, elutriate, and dosed, filtered Lake Michigan water. The percentage of freely dissolved, bioavailable contaminant in these experiments was dependent on the method of separation, the media tested, the amount of sediment/contaminant contact time, and chemical and physical properties of the individual contaminants studied. Thus, an accurate estimation of the bioavailable fraction of contaminant could not be based on simple partitioning of individual compounds.

Selective feeding habits of one indicator species, *Diporeia* spp., were examined in a series of experiments that determined the influence of sediment particle sizes on assimilation and accumulation of contaminants represented by two classes of hydrophobic contaminants. Accumulation of hexachlorobiphenyl (HCBP) was consistently greater than benzo(*a*)pyrene (BaP), suggesting a greater bioavailability of the polychlorinated biphenyl to *Diporeia*. Sediment analysis indicated that BaP and HCBP were associated with different particle-size fractions, and that these contaminants did not partition similarly to organic carbon. These data indicate that simple organic carbon partitioning models cannot be applied to hydrophobic contaminants possessing approximately the same octanol:water partition coefficients.

Other feeding studies showed differential exposure of hydrophobic contaminants could be brought about by providing uncontaminated food to organisms during bioassays. These exposure differences appeared to be compound-specific, since accumulation of some contaminants (*i.e.*, cyclodiene insecticides) was not affected upon feeding, while PAH accumulation varied with feeding.

Each of these studies showed that the bioavailability of hydrophobic contaminants depended on a number of variables, including characteristics of the indicator species, the contaminant, and the media used. The data also show that, although normalizing bioaccumulation to the amount of organic carbon in a source compartment may adjust for bioavailability differences of some contaminants, such a normalization does not adequately describe the differential bioavailability seen with other neutral hydrophobic organic compounds. Therefore, the bioavailability of these sediment-associated contaminants cannot be accurately predicted in bioassays that expose organisms to aqueous representations of whole sediment.

ACKNOWLEDGMENTS

I would like to thank the many individuals at the Great Lakes Environmental Research Laboratory (NOAA), in Ann Arbor, Michigan, for the support they have given me in this project. Specifically, I would like to thank L. Herche, who was instrumental in helping with statistical problems, and D. Gossiaux, who maintained and procured animals for this work. I also am grateful for the support of M. Lydy and J. Kukkonen, who encouraged me and provided ideas for my research during my two years at GLERL.

I would like to thank my Committee members for their participation during the course of this work, Dr. S. J. Klaine, Dr. P. Adler, Dr. T. LaPointe, and Dr. M. Hooper.

Funding for this project was provided by the U. S. Department of Commerce (NOAA), an interagency agreement supplied by the U. S. Environmental Protection Agency (#DW13935650-010), and a postdoctoral research award provided by the U. S. Air Force Office of Scientific Research through the Society of Environmental Toxicology and Chemistry, awarded to Dr. M. Lydy.

Finally, I wish to thank Dr. Peter Landrum for his assistance in this endeavor. I am grateful for the many extensive, and sometimes grueling discussions that have helped me gain insight in the field of environmental toxicology. Pete was always ready to take the time to listen and comment on my many questions and problems, and without his help, I would not have been able to complete this work.

source for Great Lakes benthos (Eadie *et al.* 1985). Accumulation of hydrophobic organics in benthos is inversely proportional to the amount of organic carbon present in the sediment (Landrum and Faust 1991), thereby creating a trade-off in the amount of contaminant available for uptake and assimilation. Amphipods of the species *Diporeia* (formerly *Pontoporeia hoyi*; Bousefield 1989) are such discriminate feeders, feeding on fine-grained sediment particles (Lydy and Landrum 1993) and accumulating a substantial fraction of their organic contaminant concentration through ingestion (Landrum 1989, Landrum and Robbins 1990). In addition to being discriminate feeders, *Diporeia* spp. are intermittent feeders, either feeding upon sediment and producing numerous fecal pellets, or not feeding (Quigley 1988). Since fecal pellets in this species are packaged inside a peritrophic membrane and individuals do not actively feed on fecal pellets (Lydy and Landrum 1993), it is relatively easy to examine and count the number of fecal pellets produced from individual animals. Therefore, it should be possible to separate the routes of exposure for *Diporeia*. Those not feeding would presumably accumulate contaminant only from interstitial water and direct contact with contaminated particles. The feeding organisms would also accumulate contaminant from ingestion. Thus, assimilation from ingested material could be determined.

The objectives were to estimate assimilation efficiencies (AEs) for a selective feeding invertebrate, to compare the AEs for a selected PAH and PCB congener simultaneously, and to examine the influence of particle size on AE. The hypotheses were that (1) assimilation efficiencies in *Diporeia* spp. could be easily calculated from tissue contaminant concentrations and feeding rates determined in animals that fed during the assays and (2) assimilation efficiencies would increase as particle-size distribution of the exposure sediment decreased. Four separate assays were conducted. First, *Diporeia* was exposed to sediment dosed with radiolabeled benzo(*a*)pyrene (BaP). The second assay was run similarly to the first, but animals were exposed for longer intervals with the addition of a second radiotracer, 2,2',4,4',5,5'-hexachlorobiphenyl (HCBP). In assays 3 and 4, the sediment was separated into different particle size fractions, < 20 μm and > 20 μm, to determine the AEs of *Diporeia* for both BaP and HCBP while, again, increasing the length of the exposure intervals.

Materials and Methods

Organisms

Diporeia spp. were collected from surficial sediment at a water depth of 29 m in Lake Michigan in the fall, spring, and summer of 1991 to 1992. Organisms were screened from the sediments, transported to the laboratory in cool lake water, and kept in 3 to 4 cm lake sediment overlaid with 10 cm lake water at 4°C in the dark. *Diporeia* spp. were allowed to acclimate for at least three days prior to the start of the assays. Voucher specimens of *Diporeia* spp. were deposited in the arthropod collection at Clemson University, SC.

Sediment

Sediment used in the assays, characterized as a silt loam soil, was obtained from Florissant, MO. This material was selected because of its low carbonate content that permitted simplification of organic carbon analysis. Its use also permitted comparison with previous efforts (Lydy and Landrum 1993), and it has been used as a reference material in previous sediment bioassays (Ingersoll and Nelson 1990). The sediment was wet-sieved through a #230 standard testing screen with lake water to obtain a particle size material of < 63 µm for use in the first and second assays. This material was further separated into < 20 µm-size particles by passing it through a 20-µm nominal pore opening Nitex screen. The material not passing through the screen was saved for use in the fourth assay. The resulting sediments were separately dosed in bulk with the radiolabeled compounds [^{14}C]-2,2',4,4',5,5'-hexachlorobiphenyl (HCBP; 12.2 mCi/mmol), and [^{3}H]-BaP (69.0 Ci/mmol) in a minimal amount (< 0.5 ml) of acetone carrier. Each compound was checked for radiopurity prior to use by a combination of thin-layer chromatography (TLC) and liquid scintillation counting (LSC; Landrum *et al.* 1987) and was > 98.0% pure. Wet sediment and lake water in a 1:1 ratio were combined, dosed, and mixed for four h at room temperature, then left to stand in the dark at 4°C for 24 h prior to beginning the assays. After standing 24 h, the overlying water, along with most of the acetone carrier, was removed before the sediment was added to the test systems. All preparative and analytical procedures were performed under gold fluorescent lights (λ > 500 nm) to avoid PAH photodegradation.

Experimental Procedure

Diporeia spp. were exposed to sediments dosed with [^{14}C]-BaP or [^{3}H]-BaP and [^{14}C]-HCBP in static systems. The first assay used a single radiolabeled tracer in Florissant soil that contained particle size fractions up to 63 µm. The second assay

used dual-labeled radiotracers, [^{14}C]-HCBP and [^{3}H]-BaP, also in Florissant soil, that contained $\leq$ 63 µm particle size fractions. The same dual-labeled radiotracers were used in the third and fourth assays. Florissant soil was separated into < 20-µm particle size fractions and > 20-µm fractions for use in assays 3 and 4, respectively.

Individual *Diporeia* were exposed in 50-ml glass centrifuge tubes that contained dosed sediment equivalent to 2 g dry weight and were filled with filtered Lake Michigan water (dosed sediment equivalent to 1 g dry weight per tube was used in assay one). Tubes that contained dosed sediment and filtered lake water were left overnight at 4°C to equilibrate before the addition of animals. One animal was placed in each tube. Tubes were individually covered with fiberglass window screening secured with a rubber band to prevent escape of the *Diporeia*. In addition, three control tubes containing contaminated sediment without animals were employed in each assay to determine any possible changes in the contaminant concentration and/or total organic carbon (TOC) over the term of the experiment. Twelve tubes that contained uncontaminated sediment with *Diporeia* spp. were also used in each assay to insure that feeding behavior was not altered by the contaminants or the dosing process. All tubes were kept in aquaria filled with aerated lake water at 4°C in the dark.

Approximately 25 tubes were sampled after each of the timed intervals (seven to 17 tubes were removed at each timed interval in the first assay). In the first and second assays, overlying water was sampled for contaminant concentration via LSC from three randomly chosen tubes after the timed intervals and was found to be not different from background concentrations. Animals and fecal pellets were removed from the sediment and prepared for LSC. Tubes were sampled after one, three, five, and seven d in the first assay, three, seven, 10 and 14 d in the second and third assays, and 10 and 14 d in the fourth assay. Feeding organisms were separated from non-feeding organisms on the basis of fecal pellet number, set at 30. This number was based on an average fecal pellet production rate reported as 10 fecal pellets $\cdot$ d^{-1} (Quigley and Vanderploeg 1991), as well as my own observations in sediment-fed *Diporeia* (*e.g.*, individuals that molted and did not feed during an assay never produced more than 30 fecal pellets). Thus, if < 30 fecal pellets were found in a tube, the organism was assigned to the non-feeder group. Fecal pellet contaminant concentrations were determined for all organisms designated as feeders. Three control tubes containing animals and undosed sediment were removed at each timed interval and were checked for organism appearance and number of fecal pellets produced.

Sediment from the tubes was combined at the end of the first and last timed intervals and analyzed for contaminant concentrations. In addition, particle mass, contaminant concentrations, and total organic carbon (TOC) were determined for the different particle-size fractions of the sediment after each timed interval in assay two. Contaminant concentrations and TOC were determined on sediment from control tubes containing dosed sediment and no animals at the end of each assay.

Sampling and Analyses

At each timed interval, individual *Diporeia* were removed from the sediment, rinsed in distilled water, blotted dry, weighed, and placed directly into xylene-based scintillation cocktail (3a70b; Research Products International, Inc.). Samples were sonicated for 30 s using a Tekmar® high intensity ultrasonic processor and were analyzed for radioactivity on a LKB 1217 liquid scintillation counter. Samples were corrected for quench using the external standards ratio method after subtracting background. Triplicate dosed sediment samples were analyzed for contaminant concentration, dry:wet ratios, and TOC content before each assay and at the end of each sampling period. The dry:wet weight ratios for sediment samples were determined by weighing a wet sediment sample and drying at 90°C to constant weight. Contaminant concentration in the sediment samples was determined by placing approximately 100 mg wet sediment directly into scintillation cocktail and sonicating the sample for two min (extraction recovery = 82 to 92% for BaP; Lydy and Landrum 1993). Fecal pellets were individually removed from the sediment via micropipet, transferred, weighed, and dried at 90°C for 2 to 3 h to constant weight. Fecal pellets were weighed again for dry:wet determinations, then placed directly into scintillation cocktail and sonicated for 60 s. Samples were left to stand in scintillation cocktail for at least 48 h before determining activity.

The TOC content of the sediment was determined by drying sediment samples to constant weight and assaying organic carbon on a Perkin-Elmer 2400 CHN Elemental Analyzer.

Fractionation of the sediment particles was determined by a modified sedimentation technique (Royce 1970, Siebert 1977). Approximately 20 g wet sediment was mixed with 1L of filtered Lake Michigan water in a 1L graduated cylinder at room temperature. Replicate 25 ml water samples were taken at 20 cm depth at 0, 120, 240, and 600 s. After 1200 and 4600 s, water samples were taken at a depth of 10 cm. The sampling times and depths were calculated by Stoke's law and used 2.6 as the specific gravity of the particles (Royse 1970). From each sample taken,

three 2 ml aliquots were analyzed via LSC. The rest of the sample (19 ml) was dried to constant weight at 90°C for mass and TOC determinations.

Calculation of Feeding Rates and Estimation of Assimilation Efficiencies

Feeding rates were calculated for individual feeding organisms (those that produced > 30 fecal pellets) as

$$FR = \frac{\text{dry weight fecal pellets (mg)}}{\text{exposure interval (h) x wet weight of animal (mg)}} \quad (1)$$

where

FR = feeding rate.

The amount of accumulated contaminant due to ingested sediment was estimated from the difference between feeding and non-feeding animals at each time interval as

$$Ca_f = Ca_t - Ca_w \quad (2)$$

where

Ca_f = contaminant concentration in organisms due to feeding ($dpm \cdot g^{-1}$ wet weight)

Ca_t = measured total contaminant concentration in organisms ($dpm \cdot g^{-1}$ wet weight)

Ca_w = estimated concentration accumulated from water (dpm/g wet weight).

Ca_w was estimated by two methods. The simplest was to use the average concentrations found in non-feeding organisms (those that produced < 30 fecal pellets) as the estimate of contaminant concentration accumulated from all routes except feeding. The second approach for estimating Ca_w was to plot Ca_t versus fecal pellet mass/organism mass for each timed interval (*e.g.*, Figure 4.1). The intercept would represent the accumulation from all non-feeding sources. This assumes that accumulation from all sources except feeding is the same whether the animals feed or not.

An assimilation efficiency (AE) was calculated for each timed interval as

$$\%AE = \frac{Caf}{FR \times SI \times t \times Cs} \quad (3)$$

where

AE = assimilation efficiency

Ca_f = mean concentration of contaminant in the animal due to feeding, as determined above (dpm·g^{-1} wet weight)

SI = selectivity index, estimated as 6.4 (Lydy and Landrum 1993)

t = exposure interval (h), and

Cs = concentration of contaminant in sediment (dpm·g dry $weight^{-1}$).

When AE is calculated from estimates of Ca_f using the difference between Ca_t and the mean of the nonfeeding group, the calculation is referred to as the mean concentration method. When the AE is calculated from estimates of Ca_f determined from the difference between Ca_t and Ca_w estimated from the regression, the method is referred to as the y-intercept method.

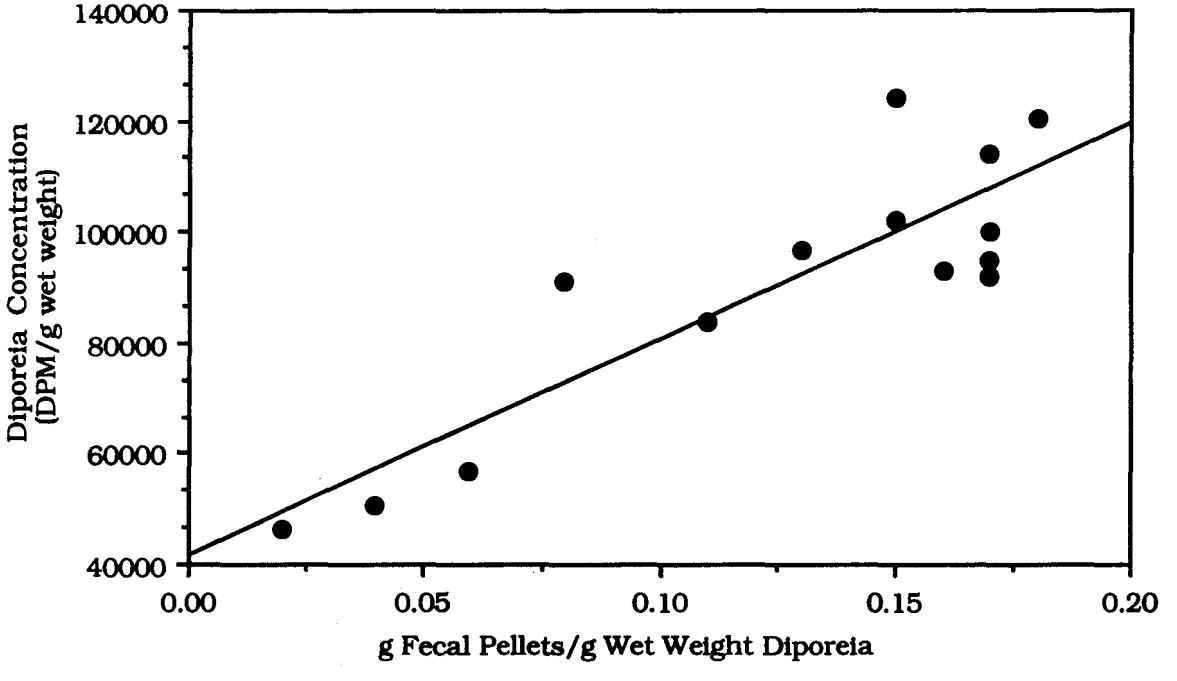

Figure 4.1. Plot estimating Ca_w using the y-intercept method. Concentration of BaP in individual animals (Ca_t) was plotted against fecal pellet mass x organism $mass^{-1}$ for data obtained at day 10 of assay four. Regression line is $y = 387{,}457x + 40{,}876$; $r^2 = 0.77$.

The selectivity index used for this work was the mean value from previous studies with *Diporeia* (Lydy and Landrum 1993) and is based on the feeding selectivity for organic carbon (Lee *et al.* 1990).

Calculation of Uptake Rate Coefficients

Accumulation data were fit to a one-compartment model by linear regression (Landrum 1989), using the equation

$$Ca_t = K_S C_S t, \quad (4)$$

where

K_S = the uptake clearance of the contaminant from sediment (g dry sediment $\cdot g^{-1}$ wet weight organism $\cdot h^{-1}$).

Two assumptions were made for the initial portion of the exposure periods (0 to 5 d). First, the concentration of contaminants in the sediment remained constant throughout the course of the assay. Second, the elimination rate and/or biotransformation of the contaminants from *Diporeia* were sufficiently slow and did not result in significant loss over the time course of the exposure. These assumptions are reasonable based on previous measures of *Diporeia* kinetics (Landrum 1989).

Statistics

Differences between feeding organisms (those that produced > 30 fecal pellets) and non-feeding organisms were determined via Student's *t* tests. Differences were considered significant when $p < 0.05$. Linear regressions were performed with the linear regression packages in SAS® (SAS 1985) and Microsoft® Excel (Microsoft 1991).

Results

No significant differences in accumulation of BaP appeared between feeders and non-feeders until day five of assay one (Table 4.1). Accumulation of BaP in assay two was lower in both feeders and non-feeders than in assay one, and significant differences in accumulation between feeders and non-feeders were apparent only after day 10 (Table 4.1). Accumulation of BaP was very low when *Diporeia* spp. were exposed to the < 20-µm particle size fraction of sediment (assay 3), and no significant differences in accumulated compound were found between feeders and non-feeders after any exposure intervals (Table 4.1). However, both a rapid increase and significant differences in accumulation of BaP were seen in assay four, where *Diporeia* were exposed to the > 20-µm fraction of sediment (Table 4.1). In assay four, accumulation of BaP was an average 4 to 17 times higher on days 10 and 14,

respectively than when organisms were exposed to all particle size fractions up to 63 μm (assay two).

Accumulation of HCBP in assay two continued to rise through day 14 in all *Diporeia*, where significant differences between feeders and non-feeders were seen after day three (Table 4.2). However, accumulation of the compound was lower in assay three, where organisms (both feeders and non-feeders) slowly accumulated HCBP to day 10, then declined to day 14. No significant differences in accumulation of HCBP were apparent between feeders and non-feeders at any exposure intervals in assay three. Accumulation of HCBP was much greater in assay four, where accumulation differences between feeders and non-feeders were readily apparent (Table 4.2). *Diporeia* accumulated an average of 4 to 6 times more HCBP in assay four than in assay two on days 10 and 14, respectively. Accumulation of HCBP was significantly greater than BaP in all dual-labeled assays (Tables 4.1 and 4.2).

Mean feeding rates calculated for each of the four assays (Table 4.3) did not follow the same trend as contaminant accumulation (Tables 4.1 and 4.2). Mean feeding rates ranged from 0.00041 to 0.00264 $mg \cdot mg^{-1} \cdot hr^{-1}$ and were highest in assay one (Table 4.3). Control animals taken from uncontaminated sediment produced fecal pellets proportional in number to test animals (*e.g.*, on day seven of assay two the three control animals produced 0, 5, and 133 fecal pellets, indicating the presence of feeding and non-feeding animals).

The concentrations of BaP and HCBP in Florissant soil did not significantly change over the time course of assays one to three, although a significant decrease in BaP concentration occurred between days 10 and 14 of assay four ($t = 18.368$, 4 *d.f.*, $P < 0.001$; Table 4.4). A large variation in sediment concentration of HCBP was observed in assay four. This may have been due to the high wet:dry ratios of the > 20-μm fraction (wet:dry ratios for individual sediment samples ranged from 1.32 to 2.78) and the difficulty of keeping the > 20-μm particles in a homogenous suspension during sampling. No significant differences in HCBP sediment concentration were seen from days 10 to 14 in assay four ($t = 0.921$, 4 *d.f.*, $P > 0.05$). Control sediments without organisms had similar contaminant concentrations as test sediments with organisms present in each of the four assays.

Uptake rate coefficients from sediment (K_S values) for accumulation of BaP in assay one were greater by a factor of two to five than for assays two and three, and generally reflected the higher feeding rates in assay one. The K_S values for BaP were comparable to previous experiments with Florissant soil as the test material (Lydy

TABLE 4.1

ACCUMULATION OF BENZO(a)PYRENE IN *Diporeia* SPP.

Assay	Days of Exposure	Number of Feeders	Accumulation in Feeders*	Number of Non-feeders	Accumulation in Non-feeders
1	1	6	0.266 (0.08)**	4	0.176 (0.07)
	3	4	0.501 (0.13)	3	0.443 (0.086)
	5	9	0.815 (0.14)	7	0.512[a] (0.084)
	7	12	1.058 (0.29)	5	0.506[a] (0.09)
2	3	6	0.387 (0.10)	18	0.302 (0.12)
	7	10	0.552 (0.13)	13	0.491 (0.18)
	10	11	0.891 (0.18)	9	0.499[a] (0.18)
	14	12	0.391 (0.20)	6	0.176[a] (0.06)
3	3	4	0.093 (0.02)	19	0.083 (0.02)
	7	11	0.208 (0.04)	8	0.181 (0.04)
	10	16	0.262 (0.07)	7	0.225 (0.04)
	14	17	0.243 (0.04)	7	0.233 (0.04)
4	10	16	4.034 (0.58)	3	2.038[a] (0.21)
	14	13	6.832 (1.73)	4	3.943[a] (1.47)

Accumulation reported as ng · g^{-1} wet weight in *Diporeia* / ng · g^{-1} dry weight sediment.

** ± 1 S. D.

[a] Significantly different from feeders at $p < 0.05$.

and Landrum 1993). Uptake coefficients for HCBP were greater than those for BaP by nearly a factor of 10. Uptake rate coefficients could not be calculated from data obtained in assay four, because only two exposure periods were examined (days 10 and 14) at intervals that did not agree with model assumptions. For assays one to three, K_S values were generated from data obtained from days one to seven only, following model assumptions.

TABLE 4.2

ACCUMULATION OF HEXACHLOROBIPHENYL IN *Diporeia* SPP.

Assay	Days of Exposure	Number of Feeders	Accumulation in Feeders	Number of Non-feeders	Accumulation in Non-feeders
2	3	5	2.199 (0.86)*	18	1.626 (0.96)
	7	6	4.368 (2.04)	14	2.695[a] (1.06)
	10	11	5.850 (1.85)	9	3.570[a] (1.84)
	14	9	7.645 (2.94)	6	4.082[a] (2.24)
3	3	4	1.884 (0.20)	19	1.837 (0.46)
	7	13	2.090 (0.44)	8	2.107 (0.36)
	10	14	3.604 (1.00)	7	3.386 (0.75)
	14	17	3.087 (0.44)	7	3.250 (0.59)
4	10	16	23.600 (3.52)	3	14.578[a] (1.43)
	14	13	46.297 (13.06)	4	30.085[a] (10.86)

* ± 1 S. D.

[a] Significantly different from feeders at $p < 0.05$.

TABLE 4.3

COMPARISONS OF UPTAKE RATE COEFFICIENTS AND FEEDING RATES FOR ASSAYS ONE TO FOUR IN FEEDING *Diporeia* SPP.

Assay	K_S BaP[a]	K_S HCBP[a]	Days of Exposure	Feeding Rate, x 10^{-3} [b]
1	0.00557 (0.0007)[c]	NA[d]	1	1.31 (0.7)
			3	2.23 (1.2)
			5	2.64 (1.3)
			7	2.30 (1.5)
2	0.00211 (0.0004)	0.01515 (0.0049)	3	1.80 (0.5)
			7	0.94 (0.5)
			10	1.24 (0.5)
			14	1.12 (1.0)
3	0.00089 (0.0001)	0.01141 (0.0010)	3	1.97 (0.3)
			7	0.84 (0.4)
			10	0.57 (0.3)
			14	0.41 (0.2)
4	NA	NA	10	0.63 (0.1)
			14	1.24 (0.6)

[a]Uptake clearance has units of g dry sed · g wet weight organism^{-1} · hr^{-1}.

[b]Feeding rate has units of mg dry weight fecal pellets · mg wet weight organism^{-1} · hr^{-1}.

[c] ± 1 S. D.

[d] Not available.

TABLE 4.4

CONCENTRATION OF CONTAMINANTS IN FLORISSANT SEDIMENT AND FECAL PELLETS IN FEEDING *Diporeia* SPP.

Assay	Days of Exposure	Sediment BaP Conc.	Sediment HCBP Conc.	Fecal Pellet BaP Conc.	Fecal Pellet HCBP Conc.
1	1	349.61 (10.5)[a]	---[b]	1071.68 (148.8)	---
	3	---	---	495.91 (139.1)	---
	5	---	---	655.18 (411.5)	---
	7	352.38 (7.2)	---	361.98 (91.6)	---
2	3	0.3917 (0.031)	350.08 (79.0)	0.4909 (0.241)	1917.84 (1616.6)
	7	0.3668 (0.012)	327.66 (53.0)	0.3236 (0.131)	1998.32 (1402.9)
	10	0.3077 (0.004)	316.06 (59.3)	0.1728 (0.063)	6776.44 (4133.2)
	14	0.3442 (0.018)	329.35 (90.2)	0.1822 (0.060)	3191.65 (2553.8)
3	3	0.2233 (0.070)	211.46 (33.0)	0.0680 (0.015)	147.02 (70.9)
	7	0.1892 (0.011)	316.26 (6.0)	0.1054 (0.017)	275.01 (70.4)
	10	0.2022 (0.003)	273.01 (9.5)	0.1129 (0.021)	298.47 (80.0)
	14	0.2357 (0.009)	383.34 (59.8)	0.0851 (0.022)	319.57 (183.3)
4	10	0.0714 (0.031)	56.97 (25.3)	0.3534 (0.077)	952.51 (260.5)
	14	0.0579 (0.031)	43.31 (4.4)	0.2452 (0.131)	686.72 (415.9)

Concentrations are given as $ng \cdot g^{-1}$ dry weight.

[a] $\pm$ 1 S. D.

[b] Experiments not performed.

In assay one, the concentration of BaP in fecal pellets (ng · g^{-1} dry weight) was three times that of the sediment concentration after day one, but dropped drastically over the remainder of exposure intervals to equal that of the sediment concentration on day seven (Table 4.4). Fecal pellet concentration of BaP followed the same trend in assay two, reaching a concentration equivalent to the sediment on day seven. After day seven, fecal pellet concentration continued to drop. However, in assay two, concentration of HCBP in fecal pellets was over five times greater than the concentration of HCBP in the sediment on day three. Fecal pellet concentration continued to rise to 21 times that of HCBP sediment concentration by day 10, then dropped at day 14 to 9.7 times the sediment concentration.

In assay three where the < 20-µm particle size fraction of sediment was used, the lower concentrations of both contaminants in fecal pellets correlated with the lower accumulation in *Diporeia* (Tables 4.1 and 4.4). The concentration of BaP in fecal pellets was consistently lower than it was in the sediment, while HCBP concentration in fecal pellets was approximately equal to that of the sediment HCBP concentration. In contrast, when *Diporeia* spp. were exposed to the > 20-µm fraction in assay four, fecal pellet concentrations of BaP were about four times that of the sediment concentration, whereas concentrations of HCBP were about 16 times that of the sediment concentration for both days of the study (Table 4.4).

Particle-size distribution of the Florissant soil used in assay two (Figure 4.2) was not significantly different among three, seven, 10, and 14 days of exposure. The 20- to 31-µm size class contained a greater portion of sediment mass than the other fractions, while the 43- to 63-µm size class contained the least amount of mass (Figure 4.2).

In assay one, TOC in the sediment dropped from 1.05 ± 0.05% on day one to 0.51 ± 0.12% on day seven. Sediment in the control tubes without animals contained 1.10 ± 0.01 % TOC at the conclusion of assay one. These results, along with the drop in fecal pellet BaP concentration after day five suggest that *Diporeia* may have used up its food supply by the end of the assay. Therefore, twice the amount of sediment used in assay one was added to exposure tubes in assays two to four. Total organic carbon dropped only slightly from 1.33 ± 0.11% on day three to 1.16 ± 0.02 % on day 14 of assay two. Control sediment without animals in assay two contained 1.34 ± 0.10% TOC. The TOC distribution of the Florissant soil used in assay two shows that the 0 to 10-µm particle size fractions contained the greatest percentage of TOC, while distributions of TOC in the larger size fractions decreased with increasing particle diameters (Figure 4.2).

The particle-size distribution for the TOC content of sediment used in assays three and four was similar to the distribution of carbon shown in Figure 4.2. Control sediment (without organisms) contained 2.14 ± 0.19% and 0.21 ± 0.01% TOC for assays three and four, respectively. Although TOC remained relatively constant for the exposure periods examined in assay four (TOC = 0.265 ± 0.06% on day 10 and 0.310 ± 0.01% on day 14), it increased slightly from 2.13 ± 0.11 % on day three to 3.41 ± 0.11% on day 14 in assay three.

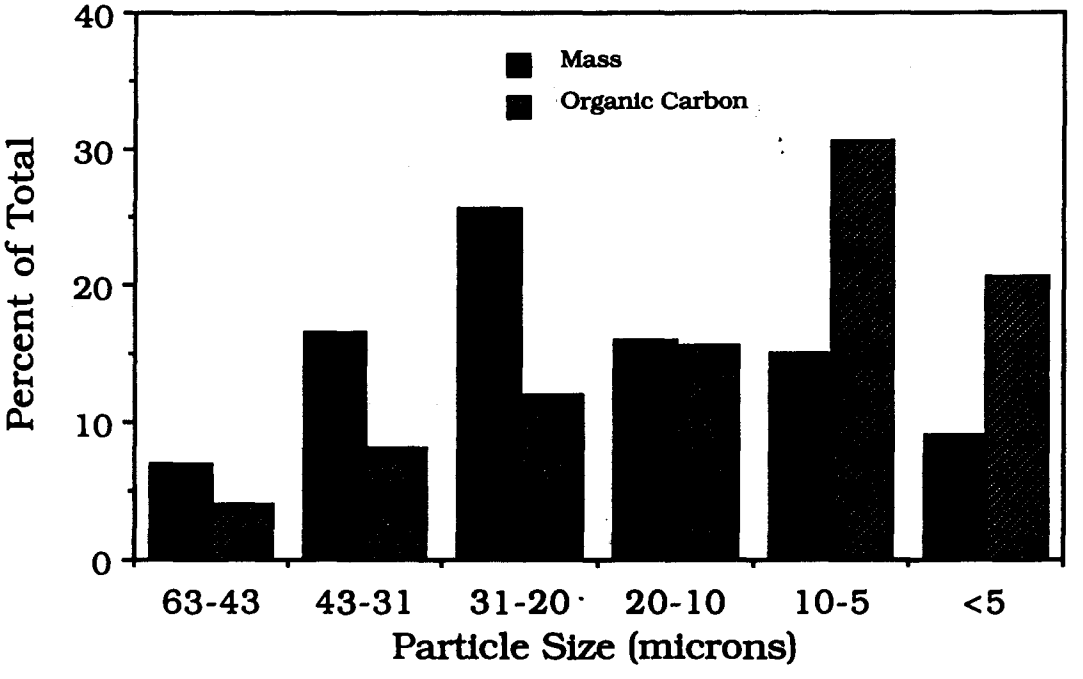

Figure 4.2. Particle mass and organic carbon distribution of Florissant soil. Values shown represent the mean of eight replicates, analyzed on days three, seven, 10, and 14 of assay two.

Contaminant distributions in the sediment for assay two suggest that BaP and HCBP distribute differently among the particle-size fractions (Figure 4.3). Benzo(*a*)pyrene tended to associate more with the < 10-µm size fractions, while HCBP tended to associate with the larger particles of 15 to 30 µm.

Since no significant contaminant concentration differences were apparent among animals designated feeders and non-feeders for many of the exposure intervals, it was not possible to calculate a value for C_{af} by the mean concentration

method (Tables 4.5 and 4.6). However, Ca_f could be determined by the y-intercept method for most of the exposure intervals which allowed AE calculation. In theory, if feeding and non-feeding animals can be accurately identified, then Ca_W calculated via the mean concentration method should approximate that obtained via the y-intercept method. Ca_W was not significantly different between the two methods. Where comparisons could be made, the two methods produce similar AE values (Tables 4.5 and 4.6). The relatively large standard deviations of contaminant concentrations and assimilation efficiencies shown in Tables 4.5 and 4.6 reflect the individual variation among *Diporeia* expressed in all of the assays.

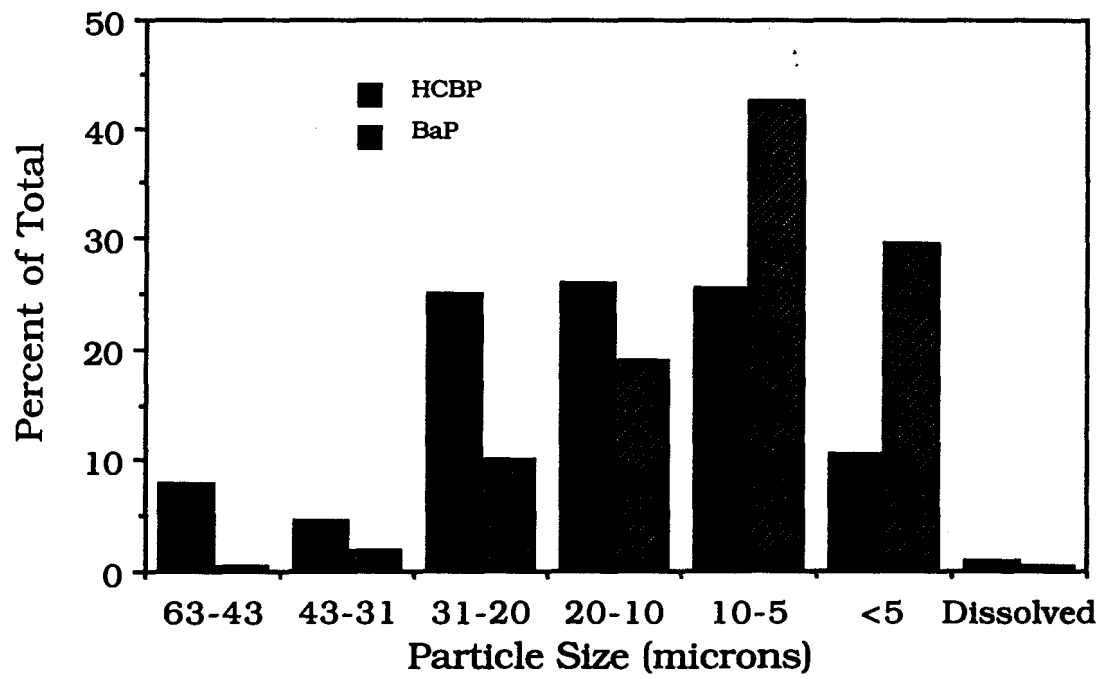

Figure 4.3. Distribution of HCBP and BaP in Florissant soil used in assay two.

Discussion

Assimilation Efficiency Determination

The technique for estimating the assimilation efficiency for BaP from the < 63 μm Florissant soil compared favorably with a previous study that examined the assimilation of BaP and calculated AE values (45.9 to 60.4%) employing TOC as a

TABLE 4.5

COMPARISON OF BaP ASSIMILATION EFFICIENCY USING MEAN CONCENTRATION AND Y-INTERCEPT METHODS

Assay	Days of Exposure	Mean Concentration Method		Y-Intercept Method	
		Mean $dpm \cdot g^{-1}$ Non-feeders, Ca_w	% BaP Assimilated	Y-Intercept, Caw	% BaP Assimilated
1	1	NA[a]	NA	6,650	29.79 (33.3)[b]
	3	13,262 (2188)	NA	10,963	14.24 (3.1)
	5	13,113 (2,420)	15.26	13,144	20.42 (14.8)
	7	NA	22.42	13,113	32.67 (14.5)
2	3	NA	NA	52,456	8.57 (3.8)
	7	54,074 (19,734)	NA	53,814	11.84 (9.0)
	10	21,288 (7,728)	17.89	88,952	5.55 (4.8)
	14	NA	15.62	97,051	NA
3	3	NA	NA	37,409	NA
	7	NA	NA	19,198	5.06 (4.3)
	10	NA	NA	19,276	11.51 (6.5)
4	10	51,199 (5,376)	207.60	40,876	238.65 (86.4)
	14	80,319 (30,008)	108.36	78,776	136.66 (88.5)

[a] Not available.

[b] $\pm$ 1 S. D.

TABLE 4.6

COMPARISON OF HCBP ASSIMILATION USING MEAN CONCENTRATION AND Y-INTERCEPT METHODS

Assay	Days of Exposure	Mean Concentration Method		Y-Intercept Method	
		Mean $dpm \cdot g^{-1}$ Non-feeders, Ca_w	% HCBP Assimilated	Y-intercept, Caw	% HCBP Assimilated
2	3	NA[a]	NA	51,010	102.69 (65.3)
	7	66,303 (26,202)[b]	164.30	9,673	278.54 (99.2)
	10	84,731 (43,713)	114.10	115,301	79.58 (74.7)
	14	100,953 (55,393)	147.01	115,042	102.88 (86.8)
3	3	NA	NA	22,650	44.15 (20.6)
	7	NA	NA	46,503	63.72 (51.8)
	10	NA	NA	42,733	174.69 (108.6)
	14	NA	NA	85,643	78.80 (96.1)
4	10	62,363 (6,142)	938.27	47,949	1171.23 (372.3)
	14	97,835 (35,325)	608.00	86,036	753.59 (654.4)

[a] Not available.

[b] $\pm$ 1 S. D.

tracer (Lydy and Landrum 1993). The AE for BaP dropped considerably in assay three, when no differences between feeding and non-feeding animals could be detected, even though feeding rate was only slightly lowered. Thus, material sorbed to the fine fraction of sediment was not readily bioavailable. The AE values for BaP in assay four were generally greater than 100%, suggesting that the source for ingestion was not accurately represented by the bulk sediment concentration. This is especially apparent when the values for assimilation of HCBP are examined for assays two and four (Table 4.6).

Because the assimilation of any compound cannot be greater than 100%, a re-examination of the terms comprising equation (3) is in order. In using this equation, AE is calculated from the concentration of compound in the animal (Ca_f) after a particular exposure period (t) to a specified contaminant concentration in the sediment (C_S). Because Ca_f is estimated from the difference of Ca_t and the estimate of Ca_W, factors that would result in an underestimate of Ca_W would make Ca_f and therefore AE high. The assumption made in this study was that the uptake for non-feeders would be representative of all the routes of accumulation except ingestion for feeding organisms. However, the act of feeding may result in more contact with particles or higher respiration that would subsequently increase the relative activity of *Diporeia*, and result in a proportionately greater accumulation from other routes than is represented by non-feeding organisms. In fact, physiological differences in some non-feeders was exemplified by the molting process. *Diporeia* that had shed their cuticle during the assays almost always produced fewer than 30 fecal pellets. When Ca_t was plotted against amount of fecal material per amount of organism, the value of the y-intercept concentration was generally not different from that measured for non-feeders. Thus, either measure is believed to be an accurate measure of the accumulation from other routes. If feeding resulted in significant changes in accumulation from other routes, then the y-intercept method would have deviated from the measured. Further, in assay three there were no differences between feeders and non-feeders, again suggesting that feeding per se does not alter the accumulation from other routes. Therefore, I assume the estimates of Ca_f are reasonable. If an error in calculating assimilation efficiency exists, it must arise in the two remaining terms, feeding rate (FR) and/or the selectivity index (SI). The feeding rate was measured based on fecal pellet production. Since feeding rate values used to calculate BaP assimilation efficiencies are the same as those used for HCBP calculations in the dual-labeled assays (assays two to four), and BaP assimilation values obtained in

assay two are indeed comparable to the values estimated in previous studies, the feeding rate term is not the likely source creating the greater than 100% AE values.

The term that is likely responsible for the error resulting in greater than 100% AE is the selectivity index, which adjusts the bulk sediment concentration to the concentration on the ingested particles. As calculated by Lee *et al.* (1990), SI is the selectivity for organic carbon (equation 5)

$$\frac{TOC_f/(1 - RC)}{TOC_S} \tag{5}$$

where

TOC_f = total organic carbon of feces (decimal equivalent)

TOC_S = total organic carbon of sediment (decimal equivalent), and

RC = reduction in carbon during gut passage (decimal equivalent).

A basic assumption for the use of the carbon-based SI value is that the contaminant will associate with the organic carbon fraction of feces and sediment. I used an SI of 6.4 to determine the AE of both BaP and HCBP, based on a mean carbon SI from a previous study that estimated the assimilation efficiency of BaP in *Diporeia* (Lydy and Landrum 1993). Although this value may be realistic for BaP assimilation in whole sediment, because the BaP distribution is similar to the carbon distribution (Figure 4.4), it appears that it must be much greater for HCBP. Part of the failure of a carbon-based SI to describe the relationship between bulk sediment and ingested sediment occurs because HCBP distribution deviates from the total organic carbon distribution in the sediment (Figure 4.4). Further, SI values ranging from 46 to 73 would need to be used to obtain a mean AE of 100% for HCBP on day 10 of assay four. Thus, the concentration on the selectively ingested particles is not determined by TOC alone, but likely includes contributions from differential partitioning and differential ingestion.

An alternative calculation would base SI simply on the ratio of contaminant concentration in fecal pellets to that in the sediment. Assuming no loss of compound during gut passage, this ratio averages 16 for HCBP in assay four (Table 4.4). This value is still well below the value of 60 needed to obtain a 100% AE alone for 10-day exposures. However, this value may be more realistic if the concentration of HCBP in fecal pellets is artificially low, which may have resulted from loss of compound when drying the fecal pellets at 90°C.

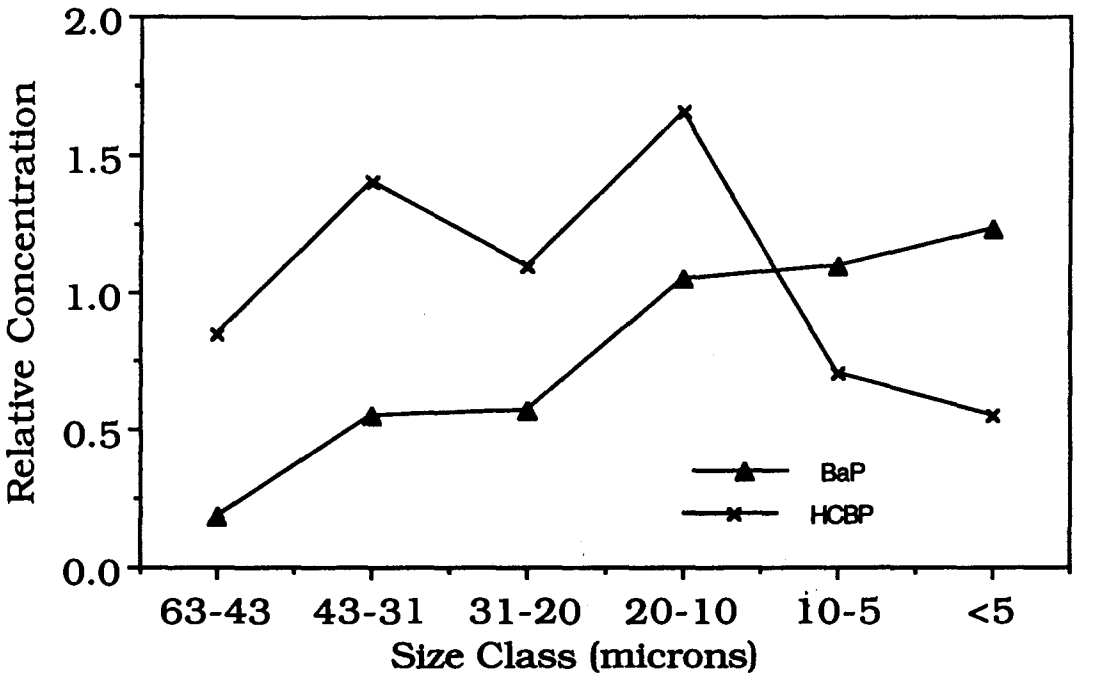

Figure 4.4. Relative organic carbon-normalized concentrations of BaP and HCBP in Florissant soil used in assay two.

Accumulation and Selective Feeding in *Diporeia*

The decrease in *Diporeia* contaminant accumulation after day 10 in assay two suggests that food resources or the bioavailable fraction of contaminants were depleted. I chose to increase the available fraction in assay three by furnishing *Diporeia* the same amount of sediment used in the previous assay (the equivalent of 2 g dry weight) that presumably contained only the "preferred" sediment fraction. According to traditional theory, deposit feeders should select smaller, high OC particles to maximize their rate of energy gain, based on the assumption that microbes are major food sources which concentrate on sediment particles (Taghon *et al.* 1978). Previous studies conducted with *Diporeia* in the lab suggested that fine-grain sediment particles of < 63-μm are selectively ingested (Landrum and Faust 1991). Other studies found that *Pontoporeia affinis*, a closely related species, selectively ingested < 10-μm size sediment particles (Moore 1976, Ankar 1977). Despite providing *Diporeia* with a presumably preferred food source in assay three, feeding rates and uptake clearances declined somewhat. Accumulation on day 10 of assay three was dramatically lower than in assay two, where feeding had a clear

influence on accumulation. Because (1) there was no difference in accumulation between feeding organisms and non-feeding organisms in assay three; (2) mass feeding rates in assay three deviated only slightly from those in assay two; and (3) fecal pellet concentrations in assay three were similar to the sediment concentration, then there was no selective feeding and the ingested material was not bioavailable.

In contrast, accumulation of both BaP and HCBP was greatly enhanced when *Diporeia* were exposed to the 20 to 63-μm size particle fractions used in assay four, compared to the ≤ 63-μm fractions used in assays one and two, and the ≤ 20-μm fraction in assay three. In addition to the enhanced accumulation, the mass feeding rate was similar to that in assays two and three, but the fecal pellet concentration was much greater than the bulk sediment concentration. These data suggest preferential ingestion among the larger particle range, and, based on the fecal pellet concentration, the preference is for particles that sorb HCBP. While the HCBP preferentially sorbs to the organic carbon in the larger fractions, this is still not sufficient to account for the concentration in the fecal pellets relative to the bulk concentration. Thus, *Diporeia* spp. must be feeding selectively within this particle size range. Futhermore, the relative concentration between fecal material in bulk sediment for the two contaminants suggests that *Diporeia* selects the particles that contain HCBP and the enhancement cannot be accounted for by the relative partitioning to the various organic carbon fractions. The selectivity for contaminants provides insight into the selectivity for nutritional requirements and suggests that *Diporeia* prefer the material on the larger particles. *Diporeia* has the ability to consume and ingest large particles, such as filamentous chains of algae with single-cell lengths of 50 μm (Quigley and Vanderploeg 1991). Finally, the decrease in concentration of both BaP and HCBP in fecal pellets from day 10 to day 14 in assay four also suggests that available food sources were depleted after day 10 in the experimental systems.

The bioavailability of hydrophobic compounds is a function of the amount of carbon in the system (Lynch and Johnson 1982, Muir *et al.* 1983, Cammen 1989, Capel and Eisenreich 1990). In this study, uptake rate coefficients declined with increasing sediment TOC (Table 4.3), consistent with previous studies (Landrum and Faust 1991). However, TOC of the sediment is not the only factor regulating the contaminant exposure in these assays. The fact that K_S values for BaP and feeding rates were both greater in assay one than in assay two, where sediment TOC was similar, may be due in part to the higher concentrations of BaP in the sediment used

in assay one. Sediment BaP concentrations were about 1000 times greater in assay one than in assay two. Increases in uptake clearances have previously been seen when *Diporeia* were subjected to sediment dosed with a mixture of PAHs ranging from 41 to 120 nmol · g^{-1} (Landrum *et al.* 1991), and when exposed to Florissant soil dosed at 1.44 nmol · g^{-1} and 42.9 nmol · g^{-1} (Lydy and Landrum 1993). The average molar concentration of BaP in the sediment used in assay one was only 1.39 nmol · g^{-1}, compared with 0.23 - 1.55 pmol · g^{-1} in assays two to four.

Exposure of *Diporeia* spp. to both contaminants was greatest in assay four, and may be due to low sediment TOC and differential partitioning of contaminants to the various particle size fractions. The large accumulation of HCBP compared to BaP in this assay may be the result of both the greater tendency of BaP to be associated with organic carbon and differential association of the contaminants to sediment particles. I have shown that realistic values for the assimilation of BaP in whole sediment could be obtained, assuming that the contaminant associates with organic carbon, while values for HCBP could not be determined using this method. Consequently, additional factors that regulate the exposure of HCBP to *Diporeia* must be involved.

The differential association of the two contaminants to particles shown in Figure 4.3 indicate that HCBP and BaP are not equally distributed among particle size classes. If this distribution was similar among particles in the 20 to 63-µm size fraction used in assay four and *Diporeia* mainly ingested particles from the larger sizes within this class (*i.e.*, the 20 to 31-µm size class), exposure to HCBP would have been greater than to BaP. Further assays that expose *Diporeia* to more extensively separated particle sizes may produce additional insights.

Conclusions

The data generated in this series of experiments demonstrated that organic contaminants that possess similar solubilities in lipid (*i.e.*, octanol:water partition coefficients) associate differently with organic carbon, which resulted in differential bioavailability to *Diporeia* spp. Although a method that bases the assimilation of contaminants on organic carbon as a tracer may be useful for some classes of hydrophobic contaminants, it is not valid for contaminants that partition differentially among organic carbon components in the system. Accumulation of the HCBP was consistently greater than the PAH in all dual-labeled sediment assays that were conducted, suggesting that the chlorinated congener was more bioavailable to *Diporeia* than the unsubstituted aromatic hydrocarbon. The greatest accumulation of

both contaminants from the 20-63 μm particle size fraction suggests that *Diporeia* prefer sediment of this size. The greater accumulation of HCBP in this size range further supports the idea that *Diporeia* exhibit an extremely selective feeding behavior. These findings should be evaluated further, using a variety of discriminate-feeding species in a range of sediment types before they are extrapolated to more natural systems.

PRELIMINARY STUDIES ON THE EFFECT OF FEEDING DURING WHOLE SEDIMENT BIOASSAYS USING *Chironomus riparius* LARVAE

Introduction

Toxicity and bioaccumulation tests are methods commonly used to determine the potential impact of sediment-associated contaminants. Various guidelines and standards for bioassays concerning the type and species of indicator organisms to be used, length of exposure intervals, and experimental design have been established (ASTM 1992*a*, *b*, U. S. EPA 1989*b*). However, there is presently a lack of consensus regarding the actual conditions for conducting sediment bioassays, such as whether to use static systems or to regularly renew overlying water, and to feed or not to feed indicator organisms during an assay. The lack of standardization has the potential to create variable and uninterpretable results among laboratories that use different protocols.

Guidelines established by the American Society for Testing and Materials (ASTM) for conducting sediment toxicity tests with marine and estuarine amphipods state that animals do not require supplementary feeding for assays of short duration, although feeding may be required for tests longer than ten days (ASTM 1992*b*). Alternatively, ASTM standards for conducting toxicity tests with freshwater invertebrates state that feeding should be included for the duration of the tests (ASTM 1992*a*), although standard food regimens, amounts, and frequencies of feeding have not been established. Further, proposed guidelines for determining the bioaccumulation of sediment-associated contaminants by benthic invertebrates recommend that food not be given during a test (U. S. EPA 1989*b*). It has been suggested that adding food during bioaccumulation tests might alter the exposure of test organisms to sediment-associated contaminants by increasing organic carbon partitioning, due to the addition of this exogenous material. Organisms that preferentially feed on food added to exposures may disrupt normal sediment ingestion and change tissue absorption by altering the physiological partitioning of contaminants within the gut (U. S. EPA 1989*b*, 1992). Feeding can also potentially alter elimination of contaminants, such that elimination may be enhanced when organisms feed on uncontaminated material. Thus, feeding of indicator species is hypothesized to reduce the accumulation and therefore the toxicity of contaminants. However, some studies have shown that feeding is necessary to avoid a high

percentage of false positives in toxicity tests, especially when nutrient-poor substrates are used (Ankley *et al.* 1993 *a, b*, Phipps *et al.* 1993).

This study was designed to determine possible accumulation effects resulting from the addition of food during whole sediment bioassays, using a variety of organic contaminants with fourth instar larvae of the midge, *Chiromomus riparius*. *Chiromomus* larvae have been recommended for use in both sediment toxicity and bioaccumulation tests (McCahon and Pascoe 1988, U. S. EPA 1989*b*) and have the potential to be used as indicator species in numerous bioassays involving hazard assessment. The objective was to temporally monitor uptake of several hydrophobic organic contaminants in organisms with and without the addition of food to determine differential accumulation of contaminants in test (fed) and control (unfed) replicates. In addition, wet weight and lipid content of animals were analyzed to determine differences between animals receiving and not receiving added food during the study.

Materials and Methods

The contaminants used in the study included ^{14}C-radiolabeled *trans*-chlordane (13.7 mCi/mmol, Velsicol Chemical Co., Memphis, TN), 4,4' [^{14}C]-DDT (11.8 mCi/mmol, Sigma Chemical Company, St. Louis, MO), [^{3}H]-benzo(*a*)pyrene (BaP, 69.0 Ci/mmol, Amersham Ltd., Amersham, UK), [^{3}H]-chrysene (340.0 mCi/mmol, Chemsyn Science Laboratories, Lenexa, KS), and [^{3}H]-pyrene (25.2 Ci/mmol, Chemsyn Science Laboratories, Lenexa, KS). All compounds were dissolved in an acetone carrier. Compound radiopurity was greater than 97% in all compounds prior to use as determined by thin layer chromatography, using either benzene:ethyl acetate (3:1, v:v, DDT and *trans*-chlordane) or hexane:benzene (8:2, v:v, BaP, chrysene, and pyrene) solvents and by liquid scintillation counting (LSC). All solvents were of HPLC grade. Analytical procedures were performed under gold fluorescent light ($\lambda \geq 500$ nm) to minimize photodegradation of the polycyclic aromatic hydrocarbons (PAHs).

Sediment was collected at a 45-m depth in Lake Michigan by Ponar grab approximately 8 km off the coast of Grand Haven, MI. The sediment was passed through a 1-mm sieve to remove debris and indigenous organisms. A sediment-water slurry was made by diluting wet sediment with Lake Michigan water in a 1:4 sediment to water ratio (w/v). Radiolabeled chemicals were added to the slurry drop by drop in a minimal amount of acetone carrier (<1 ml/L wet sediment) while being stirred on a mechanical stirrer at room temperature for four hours. Two sediments

were prepared with dual-labeled compounds in the following combinations: BaP/*trans*-chlordane and DDT/chrysene. One sediment was single-labeled with pyrene. After stirring, sediment slurries were left to settle at 4°C for 48 hours. After settling, overlying water was decanted and the sediment was washed with another four volumes of lake water. The mixture was again stirred by mechanical stirrer at room temperature for another four hours, then left to settle in the dark at 4°C for one week (first DDT/chrysene assay), one month (pyrene assay), three months (second DDT/chrysene assay), and four months (*trans*-chlordane/BaP assay) before testing.

C. riparius were reared in the laboratory at ambient temperature (21 - 23°C) on a substrate of shredded brown paper towels and a diet of ground Tetramin® (TetraWerk, Germany) and Cerophyl® (AgriTech, Kansas City, MO), according to ASTM guidelines (ASTM 1991*a*). Fourth instar larvae were removed from the culture aquaria and placed in environmental chambers with the same photo period (18D:6N) and feeding schedule as in the original culture aquaria. All exposures in this study were conducted at 10°C to slow larval development, so that adult emergence would not resume during the assays. Larvae were acclimated to 10°C by lowering the temperature by not more than 2°C in a 24-h period. All feeding studies were conducted at 10°C in an environmental chamber using a 18D:6N photoperiod.

Static exposures consisting of 30 g wet sediment overlaid with 20 ml Lake Michigan water in 50 ml beakers were prepared and allowed to settle overnight before adding animals. Two fourth instar *C. riparius* larvae were added to each beaker for timed intervals of 48 h, 96 h, seven d, and ten d. Two sets of feeding exposures were created along with one set of control exposures. Two mg Cerophyl® in a water suspension were added to each Level I test beaker every third day of the assay. This dose was doubled for Level II test beakers. These feeding levels were comparable to those used in previous whole-sediment assays that employed midge larvae (Nebeker *et al.* 1984, Ankley *et al.* 1993*a*). Control beakers received no additional food. Five replicate exposure beakers from each of the two feeding levels and control groups were sampled at the completion of each exposure interval. Individual larvae were removed from the sediment, rinsed in distilled water, blotted dry, weighed, and placed into xylene-based scintillation cocktail (3a70b; Research Products International, Inc., Mt. Prospect, IL) for direct extraction of the contaminants. Since previous elimination studies that tested fourth instar *C. riparius* larvae at 10°C showed that only a minimal amount of the contaminant was associated with gut contents, the organisms were not purged of gut contents before analysis. After standing 48 h in the cocktail, larvae were analyzed via liquid

scintillation counting (LSC). Lipid content of individual animals was analyzed after two-, four-, seven-, and ten-d exposures for two of the assays (pyrene and the second DDT/chrysene assays) to determine any differences in lipid accumulation between test and control exposures, using micro-gravimetric procedures (Gardner *et al.* 1985).

Contaminant concentration and total organic carbon (TOC) in sediment from each test group and controls were also determined after each exposure interval. Sediment samples were weighed and dried at 90°C to constant weight for wet:dry weight ratios. Contaminant concentration in the sediment samples was determined by placing approximately 100 mg wet sediment directly into scintillation cocktail and sonicating the sample for two min, following the procedure of Lydy and Landrum (1993). All samples for LSC analysis were left to stand in scintillation cocktail for at least 48 h before determining activity. Samples were corrected for quench by using the external standards ratio method after subtracting background. The TOC content of sediment samples was determined by drying the sediment to constant weight, treating with 1 *N* HCl to remove carbonates, redrying, and assaying organic carbon on a Perkin-Elmer 2400 CHN Elemental Analyzer.

Differences in larval weight and contaminant accumulation between controls and the two feeding levels were determined for each timed interval by using Student's *t* tests. Differences were considered significant when $p < 0.05$.

Results

Apparent steady-state concentrations of contaminants in larvae were attained prior to 240 hours in all of the assays. Steady-state concentrations in control exposures were apparently reached by 48 hours for *trans*-chlordane (Figure 5.1) and chrysene (Figure 5.2). Accumulation of pyrene in controls approached steady state by 168 hours, but decreased at 240 hours (Figure 5.2), although mean Feeding Level I accumulation appeared to attain steady-state by 48 hours. Larval accumulation of DDT and BaP continued to rise in controls and in both feeding levels throughout the studies (Figures 5.1 and 5.2), with the exception of control BaP exposures, where steady-state levels were attained by 168 hours. DDT accumulation was greater from sediment stored one week than from sediment stored three months (Figure 5.1), even though DDT concentrations in the two sediments were similar (Table 5.1). However, accumulation of chrysene, the dual-labeled counterpart of DDT in the same sediments, was similar in both assays (Figure 5.2). Larval accumulation was proportional to contaminant concentrations found in the sediments (Figures 5.1 and 5.2, Table 5.1). Mean bioaccumulation ratios for controls (ng contaminant /g wet

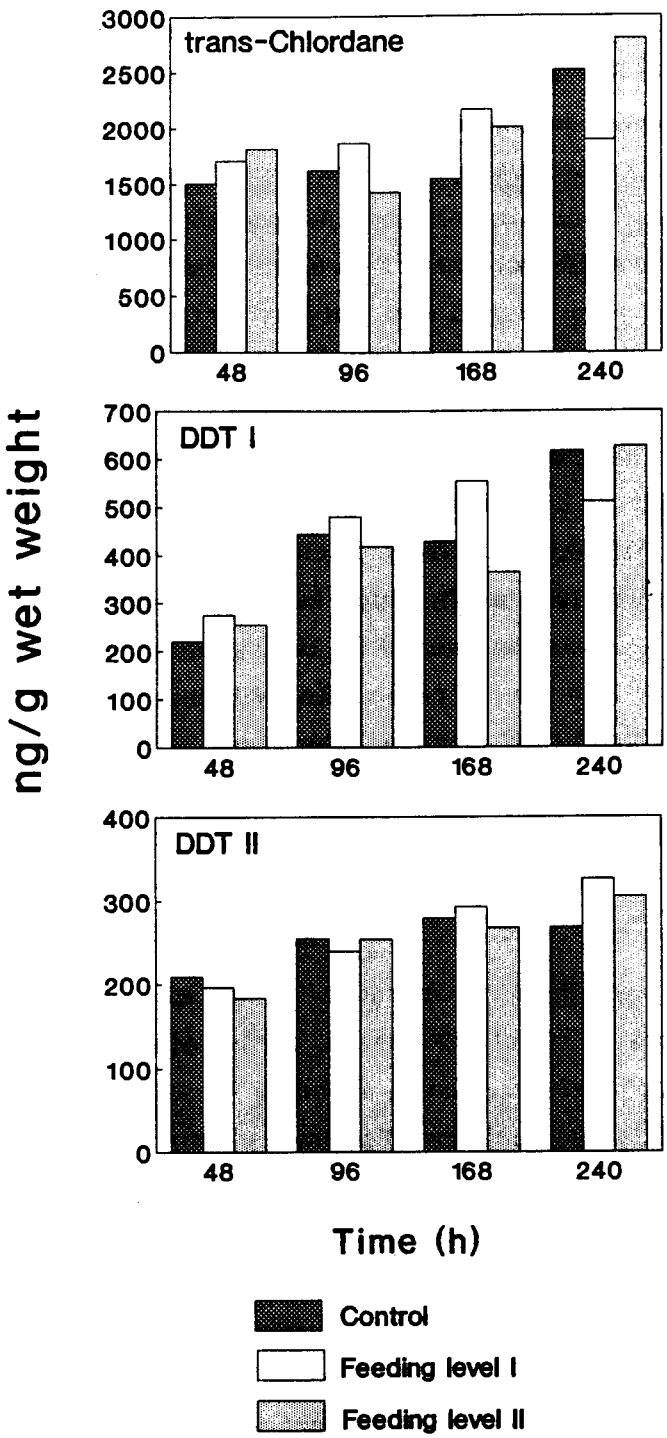

Figure 5.1. Mean concentration of *trans*-chlordane and DDT (two separate exposures) in *C. riparius* larvae after 48-, 96-, 168- and 240-hour exposures in whole sediment. Control = no food added, feeding level I = 2 mg food added per beaker, and feeding level II = 4 mg food added per beaker. N = ten larvae for each exposure interval.

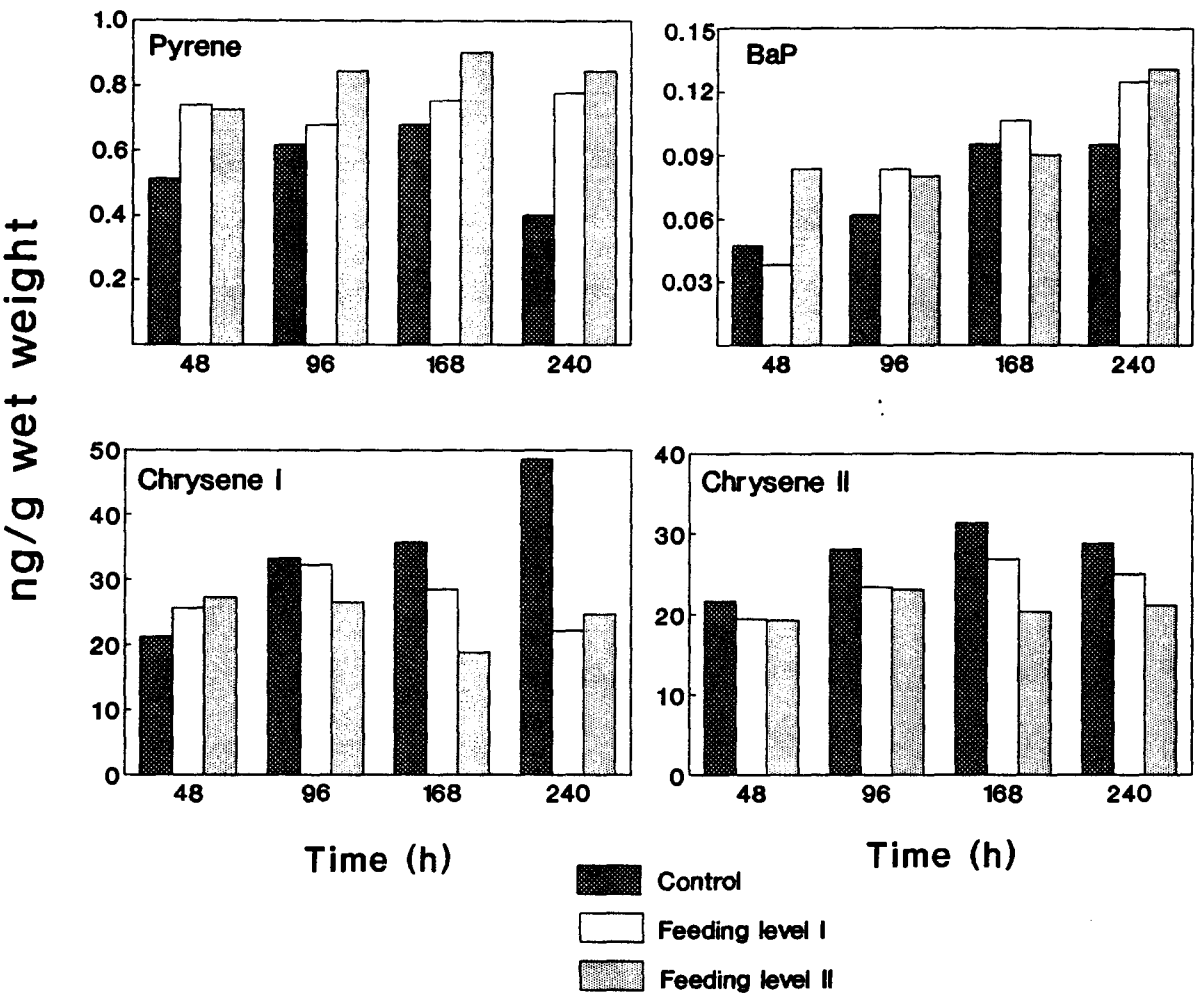

Figure 5.2. Mean concentration of pyrene, benzo(*a*)pyrene, and chrysene (two separate assays) in *C. riparius* larvae after 48-, 72-, 168-, and 240-hour exposures in whole sediment. Control = no food added, feeding level I = 2 mg food added per beaker, and feeding level II = 4 mg food added per beaker. N = ten larvae for each exposure interval.

TABLE 5.1

CONCENTRATION OF CONTAMINANTS IN *Chironomus riparius* LARVAE AFTER SEVEN-DAY EXPOSURES IN WHOLE SEDIMENT

Contaminant	Concentration of contaminant in sediment, ng/g dry weight	Control ng/g wet weight	Feeding Level I ng/g wet weight	Feeding Level II ng/g wet weight
Pyrene	0.640	0.6819	0.7549	0.8638
	(0.06)*	(0.298)	(0.285)	(0.320)
BaP	0.269	0.0451	0.1063**	0.0902**
	(0.01)	(0.013)	(0.040)	(0.018)
Chrysene I	32.400	35.67	28.53	18.83**
	(7.06)	(10.9)	(13.7)	(4.2)
Chrysene II	33.171	31.32	26.80	20.31**
	(2.60)	(6.6)	(7.7)	(5.9)
DDT I	381.93	428.42	553.58	364.24
	(66.43)	(140.4)	(195.8)	(125.3)
DDT II	349.82	279.51	292.91	267.96
	(53.72)	(53.89)	(103.0)	(72.0)
trans-Chlordane	1016.21	1547.53	2170.40	2008.82
	(72.22)	(968.7)	(289.0)	(506.0)

* $\pm$ 1 S. D.

** Values are significantly different from controls at $p < 0.05$.

weight larvae : ng contaminant/g dry weight sediment) ranged from 0.63 (pyrene) to 2.48 (*trans*-chlordane) after 240 hours.

Contaminant accumulation in larvae differed significantly between controls and feeding levels for PAHs, but was generally not consistent among the exposure intervals. Larvae in both feeding levels accumulated significantly more pyrene than controls after 48 and 240 hours ($p < 0.05$), but no significant differences were seen among feeding levels and controls at 168 hours ($p = 0.296$ and 0.217 for controls and Feeding Levels I and II, respectively; Table 5.2). BaP-dosed larvae showed significantly higher accumulation in both feeding levels than in controls only at 168 hours (Table 5.2). Conversely, larvae exposed to chrysene-dosed sediments in fed exposures accumulated less contaminant than controls after 96 (Study II), 168 (Studies I and II), and 240 hours (Studies I and II; Table 5.2). Accumulation was not significantly different among either of the feeding levels or controls for the two insecticides studied. Small differences in individual uptake rates and/or physiological state of the larvae may account for the significant differences in accumulation of DDT between the two feeding levels at 168 hours in the first DDT/chrysene assay ($t = 2.494$, 16 df, $p = 0.013$; Table 5.2).

Wet weights of control larvae were not significantly different from test larvae at the completion of the assays, except for pyrene exposures. In single-labeled pyrene exposures, Feeding Level I and II larvae were an average 41.0 and 66.0% heavier than control larvae after 240 hours ($t = 2.680$, 16 df, $p = 0.0014$, control/Feeding Level I; $t = 2.812$, 16 df, $p = 0.0078$, control/Feeding Level II). Average larval wet weights in all assays rose from 9.64 $\pm$ 2.2 mg (mean $\pm$ 1 S. D.) at 48 hour to 12.84 $\pm$ 1.2 mg at the completion of the assays.

Lipid content in larvae remained similar throughout the exposure intervals and was not significantly different among either the feeding levels or controls. Larval lipids, sampled from pyrene and the second set of DDT/chrysene assays, averaged 2.99 $\pm$ 1.3%, 2.93 $\pm$ 1.3%, and 3.39 $\pm$ 1.7% on a dry-weight basis for Feeding Levels I, II, and controls, respectively. Dry to wet weight ratios for *C. riparius* larvae averaged 3.5.

Survival of control larvae in all assays was 100%. Survival in Feeding Level I was lowest, with 87.5% of the larvae surviving to 240 hours in the BaP/chlordane exposures, 95% surviving in pyrene and the first DDT/chrysene exposures, and 80% surviving in the second DDT/chrysene exposures. All larvae survived to 240 hours in Feeding Level II exposures except with BaP/chlordane exposures, where survival was 87.5%.

TABLE 5.2

DIFFERENCES IN CONTAMINANT ACCUMULATION BETWEEN FEEDING LEVELS I, II, AND CONTROLS, USING STUDENT'S *t*-TESTS FOR EACH TIMED EXPOSURE INTERVAL

	48 Hours		96 Hours		168 Hours		240 Hours	
Contaminant	A	B	A	B	A	B	A	B
Pyrene	+	+	0	+	0	0	+	+
BaP	0	0	0	0	+	+	0	0
Chrysene I	0	0	0	0	0	-	-	-
Chrysene II	0	0	-	-	0	-	0	-

A = comparison between controls and Feeding Level I, B = comparison between controls and Feeding Level II. All data were considered significant at $p > 0.05$. 0 = no significant difference between categories; + = accumulation significantly greater than found in controls; - = accumulation significantly lower than in controls (*e.g.* + in Column A indicates Feeding Level I accumulation was significantly greater than in control exposures.) No differences between feeding level I and feeding level II were found for any of the compounds studied at the $p < 0.05$ level of significance.

Because the amount of exogenous food added to the exposures was quite minimal, sediment total organic carbon did not change over the course of any of the assays and ranged from 0.42 to 0.53%. No trends in sediment contaminant concentrations were observed, and contaminant concentrations in sediment remained the same for all contaminants over the course of the exposures.

Discussion

Feeding the test organisms during a bioassay was expected to reduce contaminant accumulation. When feeding is a dominant route for contaminant accumulation, changes in organism health in response to the contaminant can act to enhance or reduce ingestion and/or elimination rates. For example, stress placed on the assay organism from transport between the culture aquaria and test exposures, or from an exposure environment unusual to the organism (*i.e.*, aqueous exposures without substrate for burrowing) may reduce its feeding rate. Conversely, feeding rate as well as overall metabolic rate may be increased as a stimulatory response to

contaminant exposure. If uncontaminated food is preferentially ingested, elimination may be enhanced from differential partitioning of the contaminants to fecal material. In addition, preferential ingestion of uncontaminated food will reduce (dilute) the effective concentration of contaminants accumulated as the organism feeds to meet its nutritional requirements. Finally, the overall feeding rate in the presence of a high quality food may reduce feeding and elimination rates due to the different nutritional content of the added food compared to sediment. Thus, it is possible to see a decrease in contaminant accumulation when organisms are fed uncontaminated food during a bioassay. This may explain the results obtained in the chrysene exposures, where feeding may have resulted in a combination of decreased contaminant ingestion and increased elimination. Previous studies showed that toxicity of sediment-associated metals to oligochaetes decreased when exogenous food was added in laboratory tests (Wiederholm *et al.* 1987). This may have been a result of lower exposure or increased elimination, as observed for chrysene, or an overall improvement in organism health.

Differential accumulation in larvae between feeding levels and controls may have been due, in part, to physical and chemical characteristics of the contaminants. Contaminant bioavailability of the two insecticides, DDT and *trans*-chlordane, did not change upon addition of food to the exposures. However, differences in accumulation between feeding levels and controls were seen after at least one of the exposure periods for BaP and pyrene. The increased bioavailability of these two contaminants with feeding may be due to enhanced sorption of these PAHs onto food particles. Feeding has led to enhanced cadmium toxicity because of rapid transfer of cadmium from test solutions onto food and food/sediment mixtures (Pascoe *et al.* 1990). Any sorption of BaP and pyrene onto uncontaminated food particles may have increased the bioavailability of these contaminants, and effectively "overridden" any reductions in contaminant ingestion resulting from decreased feeding rates, or increases in elimination due to contaminant effects (*e.g.*, higher metabolic rate).

The significant difference in accumulation of DDT between sediment aged one week and three months indicates that the bioavailability of DDT decreased with sediment aging, while that of chrysene dosed in the same sediment stayed relatively constant. The chrysene data agree with previous studies that reported uptake clearance values for chrysene in *Diporeia* spp. not significantly different between sediments spiked three days and 150 days prior to exposures (Landrum *et al.* 1992*c*). Other studies have reported bioavailability of PAH from sediment to significantly decline with increased contact time between the sediment and contaminants

(Varanasi *et al.* 1985, Landrum 1989). The reasons for the variability among these studies are not known, and no similar studies have examined changes in DDT bioavailability with sediment aging. In this study, the concentration of DDT was an order of magnitude greater than that of chrysene in the dual-labeled sediment. These concentration differences may have affected contaminant partitioning over the three month aging period. Further studies relating the bioavailability of sediment-associated contaminants with sediment aging will need to be undertaken before an understanding of this phenomena is attained.

Larval feeding selectivity may have accounted for the variation in contaminant accumulation. Although *Chironomus* larvae are described as filter feeders, feeding on algae and detritus (Walshe 1951, Coffman 1967, Oliver 1971), very little is known about their feeding preferences. Differential sorption of the contaminants to food or sediment particles, coupled with selective ingestion of these particles, may significantly influence larval exposure, as shown in previous studies that examined the uptake of hexachlorobiphenyl and BaP in a benthic amphipod (Harkey *et al.* 1993*a*).

Although the quantity of food added to the exposures in this assay was comparable to that used in some previous studies, it was much lower than amounts used in other studies. I used concentrations of 0.022 and 0.044 mg food per ml wet sediment per day for Feeding Levels I and II, respectively. Ankley *et al.* (1993*a*) reported that optimal conditions for second instar *C. tentans* larvae in toxicity assays were obtained with the addition of 0.038 mg food per ml wet sediment per day. "Optimal" conditions were specified as those resulting in the highest survival of larvae with minimal loss of water quality. Other studies have reported adding up to seven times this amount of food to exposures (Pittinger *et al.* 1989, Ingersoll and Nelson 1990). Food concentrations at these levels have the potential to lower water quality, alter organic carbon content of the substrate, and change the bioavailability of the contaminants being tested.

An additional factor to consider in feeding studies such as these is the quality of food available to the test organisms, both that contained in the exposure sediments and that added exogenously. A major factor influencing the results of studies that examined growth, survival, and reproduction of species exposed to sediments differing in organic carbon content, particle size distribution, and chemical composition was nutrition (Wiederholm *et al.* 1987, Ankley *et al.* 1993 *a, b*, Phipps *et al.* 1993). In general, these studies demonstrated lower growth rates, reproduction, and survival in nutritionally poor substrates such as sand and

sediments from oligotrophic lakes. Thus, it is suggested that food be added to nutritionally poor sediments during bioassays in order to avoid results that confound nutritional quality with contaminant toxicity (Phipps *et al.* 1993). However, the low organic carbon content of the sediment in this study (approx. 0.47%) appears to have had little effect on growth and lipid content of the organisms.

Published guidelines for conducting toxicity assays with *Chironomus* spp. recommend that first to second instar larvae be used (ASTM 1991*a*). Fourth instar larvae were used in this study due to the ease of identifying and handling the organisms. However, individual *Chironomus* sp. in fourth larval instars may be at very different stages of development (Wülker and Götz 1968). The variation in contaminant accumulation, especially that noted between 168-and 240-hour exposure periods, may be attributed to changes in physiology and metabolic rates among the groups of individuals used in this study. In addition, significant differences in accumulation of contaminants between fed and unfed animals in bioassays will change with the species and life stage used, as well as with the temperature at which the assay is conducted. Most midge studies have been run at room temperature; therefore, the results of this study cannot accurately be extrapolated to previous assays conducted at 22 - 25°C. Any statistically significant differences in contaminant accumulation may be more or less marked under different environmental conditions. Consequently, the relative magnitude of such statistical differences becomes a point of concern with the development of standard bioassay procedures. Further research will be needed to describe the environmental conditions (*e.g.*, temperature, organism density, sediment volume) that most favorably define such endpoints as accumulation, growth rate, and weight gain.

Bioaccumulation models of hydrophobic organic contaminants have made the assumption that compounds will partition to organic matter in proportion to their respective hydrophobicities, as measured by log K_{OW}. However, most bioaccumulation models assume that partitioning of contaminants is at equilibrium during the time of exposure. If equilibrium is attained rapidly (*e.g.*, partitioning to added food particles happens quickly), then the behavior of hydrophobic non-polar compounds should behave consistently in proportion to the hydrophobic nature of the contaminant. The preliminary data from this study did not exhibit consistency. Rather, the compounds behaved differently both between compound classes (chlorinated hydrocarbons and PAHs) and within a compound class (PAHs). The inconsistencies among compounds do not exhibit any particular pattern with respect to the hydrophobicity of the compound, so hydrophobicity alone is insufficient to

explain the variance either within the PAH group or between compound classes. The absence of simple partitioning relationships to explain the differential accumulation of hydrophobic contaminants leaves one to speculate about the mechanisms and processes that led to these data. Potential explanations include differential partitioning among particle types, selective ingestion, and alteration of accumulation and elimination kinetics. Certainly, further research on the route of exposure and the partitioning among particles will aid in understanding the exposure potential for various compounds. Until the factors that influence the variance in exposure are identified and can be accounted for, the influence of feeding in toxicity bioassays will make the exposure, and therefore the results, poorly interpretable.

CONCLUSIONS AND SUGGESTIONS FOR FUTURE RESEARCH

Based on data from the studies presented, the three hypotheses stated early in this thesis can now be addressed. To reiterate, these hypotheses were:

1. Exposure to hydrophobic organic contaminants in whole sediment will differ from porewater and elutriates produced from it.
2. Accumulation of sediment-associated contaminants will vary among species possessing different feeding behaviors and life histories.
3. The exposure represented by a number of hydrophobic organic contaminants in porewater and elutriates can be explained by comparing the proportion of contaminants bound to organic and inorganic material to that freely dissolved in the aqueous phase.

The studies that compared clearances and accumulation of hydrophobic contaminants to indicator species in whole sediment, porewater, and elutriates support the first hypothesis. In most cases, porewater and elutriates greatly under-exposed organisms, even after adjusting for the amount of organic carbon in the exposure media. Consequently, organisms in whole sediment accumulated contaminant more rapidly and to a greater extent than those in aqueous media. The greater exposure in whole sediment was, most likely, due to multiple exposure sources (*i.e.*, ingestion of sediment solids plus ventilation of porewater), compared to single exposure sources available from aqueous media.

The differential accumulation of contaminants exhibited by the three indicator species used in the comparison studies also supports the second hypothesis. In most cases, *C. riparius* accumulated less contaminant than either *Diporeia* spp. or *L. variegatus*; accumulation in *Diporeia* spp. was usually the greatest for a given length of exposure. These comparison data also demonstrate the importance of sampling time in bioassays. In the few instances where accumulation was comparable among elutriate or porewater and whole sediment, the exposure intervals over which accumulation was the same in aqueous media and whole sediment varied with the species used. For example, accumulation of pyrene from elutriate was not significantly different from accumulation in whole sediment up to 48-h exposure intervals in *L. variegatus*. After 48 h, *L. variegatus* showed significantly more accumulation from elutriate, while *C. riparius* showed significantly less accumulation from elutriate than from whole sediment. However, *Diporeia* spp. continued to accumulate the same level of pyrene in elutriate and whole sediment exposures through the end of the sampling intervals (2 weeks).

The third hypothesis is not supported by the studies presented here. Data from elutriate/porewater assays suggest that, although contaminant partitioning to organic carbon may approximate the bioavailability of some hydrophobic contaminants (*i.e.*, compounds possessing log octanol:water partition coefficients of around 6), it fails to explain the bioavailability of other, more soluble contaminants (*e.g.*, pyrene, endrin). In a few cases, even accumulation of the relatively insoluble contaminants did not follow the pattern that a simple organic carbon partitioning model would suggest. For example, partitioning of BaP (log K_{OW} = 6.5) was consistent in porewater and elutriates, regardless of sediment/contaminant contact time, with about 95-100% of the total contaminant in the bound phase. Although very little of the total contaminant was available for uptake in the aqueous exposures, this amount should have been similar for all species in elutriates and porewater. Accumulation from these exposures was similar over each sampling interval for *C. riparius* and *Diporeia* spp. However, BaP accumulation was much greater in porewater exposures in one *Diporeia* assay and the *L. variegatus* assay, reported in the appendix. That these accumulation values from porewater were generally more than twice those from elutriates for the same exposure periods indicates that the freely dissolved fractions of BaP cannot always explain the differential accumulation of even a relatively insoluble contaminant.

A number of factors are responsible for the differential accumulation and uptake clearances observed among species for various contaminants and exposure media. These factors include (1) variation in species behavior, (2) mode of contaminant uptake to include feeding, (3) physical and chemical properties inherent to individual contaminants that affect partitioning to organic material, (4) changes in organic carbon partitioning with sediment/contaminant contact time, and (5) physical variation in the exposure media (*i.e.*, aqueous vs. whole sediment). The interrelationships of these factors for single contaminants are unknown. Therefore, aqueous fractions of whole sediment do not accurately represent the exposure from whole sediment, and that such assessments that extrapolate bioaccumulation potential from aqueous extracts are not accurate.

Data from the studies presented cast doubt on using whole sediment in bioassays to estimate the bioavailability of in-place contaminants. In most cases, bioaccumulation of individual contaminants in whole sediment of the same composition and organic carbon content varied greatly among the species as well as among the contaminants used. Particle-size distribution analysis in the *Diporeia* spp. assimilation study indicated that the bioavailability of contaminants such as

BaP and HCBP are differentially associated with different particle size fractions, with BaP having the greater tendency to associate with total organic carbon. These data may help explain the variation in species accumulation among the contaminants used in the comparison studies. The differential accumulation observed with some contaminants in the feeding studies also suggests that bioavailability of hydrophobic contaminants cannot be explained by simple partitioning to organic carbon alone. In feeding assays, accumulation of contaminants was not consistent among test organisms (food added during the assay) and controls (no food provided). Thus, the partitioning of contaminant on to or off of food particles was apparently compound-specific, and not broadly predictable. If predictable results cannot be derived from controlled, laboratory experiments with sediments of known organic carbon content and chemical composition, then bioavailability extrapolations to a natural environment where such factors as chemical loading, temperature, current flow, and bioturbation are constantly changing cannot be accurately estimated.

The many problems in assessing the availability of contaminants to biota with whole sediment and aqueous fraction testing apparently make laboratory bioassays a less than accurate approach to assessing contaminated sediments. Bioassays that use whole sediment must use sediment dilutions with reference sediment, that, in itself, poses problems of what reference sediment to use and the length of mixing and equilibration time prior to the start of bioassays. Sediment extracts generally under-estimate bioavailability of hydrophobic organic contaminants. Certainly a new approach is needed that accurately addresses the hazard represented by sediment-associated contaminants in the environment. Since bioavailable contaminant concentrations in sediment and interstitial water cannot be accurately determined at this time, contaminant concentrations from these sources are useless to describe effects potential on the aquatic community.

An alternative to determining dose-response effects from source compartments may be to determine whole body toxicant residue in benthos taken from areas of concern. For hydrophobic organic narcotic chemicals, whole body residue levels associated with threshold LC_{50} values (those concentrations that are expected to kill 50% of the population) have been shown to be consistently in the range of 2 - 8 mmol/kg (McCarty 1990). An extrapolation of effects-level tissue concentrations for this class of compounds could theoretically be constructed to estimate the extent of contamination in any area where benthos could be sampled for tissue residue analysis. Such an approach may be useful only after compilation of a

large data base that would relate tissue residue levels to effects, such as decreased growth, reproduction, or increased mortality in benthic populations.

APPENDICES

Appendix A

Accumulation of Contaminants in Indicator Species[a]

TABLE A-I

trans-CHLORDANE ACCUMULATION IN *Chironomus riparius*

Mean Sediment Concentration: 1,663.0 ng/g dry weight (assay I; aged 1 week)
1,740.6 ng/g dry weight (assay II; aged 2 months)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	1,110.0	287.6**[c]	163.8**	523.4	134.0	150.7**
	(256)[b]	(350)	(63)	(267)	(54)	(21)
6	2,952.6	667.4**	287.3**	1,639.7	518.0	440.8**
	(773)	(253)	(57)	(366)	(173)	(122)
24	8,227.6*	1,970.0**	908.8***	5,920.0	2,066.9	1,146.6**
	(3,158)	(217)	(331)	(948)	(321)	(206)
48	14,735.8	1,926.5**	620.1**	5,984.3	2,241.2*	1,004.2**
	(3,672)	(367)	(186)	(1,621)	(939)	(217)
72	12,651.6	2,975.8**	560.8***	7,297.2	2,458.3	1,327.7**
	(4,865)	(551)	(121)	(1,900)	(476)	(336)
96	16,224.1	2,561.8***	511.5***	8,417.0	3,327.3	877.6***
	(3,566)	(981)	(147)	(2,050)	(1,273)	(271)
K_{uoc}[c]	358.0	66.6	80.1	233.5	24.9	40.4
	(25)[d]	(6)	(15)	(16)	(2)	(3)

TABLE A-I (Continued)

Compound: *trans*-Chlordane
Species: *Chironomus riparius*
Mean Sediment Concentration: 1,813.8 ng/g dry weight (aged 6.5 months)

	Assay III		
Hours	Sediment	Elutriate	Porewater
6	1,526.4	789.3	529.1**
	(300)	(96)	(125)
24	4,031.3	1,734.0	1,707.2**
	(519)	(299)	(215)
48	4,390.6	2,779.0	2,989.9**
	(912)	(462)	(190)
72	5,887.7	3,463.1	3,018.2**
	(522)	(1,009)	(702)
96	6,213.9	2,903.6	3,082.9**
	(638)	(877)	(692)
K_{uoc}	139.1	60.9	60.5
	(13)	(6)	(4)

[a]Accumulation values were calculated as

$$\frac{\text{µg contaminant/g wet weight animal}}{\text{ng/ml contaminant in media} \cdot \text{ng/ml total organic carbon in media}^{-1}}$$

[b] ± 1 standard deviation

[c]Units for K_{uoc} are : $\frac{\text{µg organic carbon cleared}}{\text{gram organism/hour}}$

[d] ± 1 standard error

[e]Mean accumulation values were determined for six individual animals. Asterisks denote cases where less than six animals were analyzed (*n=5, **n=4, ***n=3).

TABLE A-II

PYRENE ACCUMULATION IN *Chironomus riparius*

Mean Sediment Concentration: 0.64 ng/g dry weight (assay I; aged 1 week)
0.64 ng/g dry weight (assay II; aged 1 month)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	677.3	401.4	322.9**	883.7	857.1**	1,753.7
	(228)	(16)	(60)	(265)	(508)	(719)
6	1,382.1	531.7	499.4	2,019.4	1,357.2**	1,794.4
	(506)	(139)	(192)	(550)	(237)	(387)
24	4,452.9	2,262.1	2,588.0**	2,908.0	3,846.4	3,189.4
	(313)	(484)	(814)	(1,213)	(2,109)	(654)
48	5,821.4	4,834.0	2,273.9**	4,132.2	5,000.5	6,328.9
	(1.088)	(1,791)	(240)	(860)	(2,025)	(1,846)
72	5,359.5	6,761.9	4,436.2***	4,792.5*	7,105.6	7,941.6
	(1,176)	(2,264)	(706)	(1,641)	(2,139)	(1,740)
96	6,594.2	6,812.9	4,626.9**	3,772.6	7.667.6	10,175.4
	(1,014)	(3,459)	(647)	(817)	(4,715)	(1,561)
K_{uoc}	165.9	61.4	85.9	227.1	55.7	131.5
	(9)	(4)	(7)	(50)	(5)	(20)

TABLE A-II (Continued)

Compound: pyrene
Species: *Chironomus riparius*
Mean Sediment Concentration: 0.87 ng/g dry weight (aged 1 week; used with endrin in a dual-labeled assay)

	Assay III		
Hours	Sediment	Elutriate	Porewater
1	1,700.3	952.5**	1,156.3**
	(454)	(187)	(856)
6	6,469.9	4,712.1**	2,088.4
	(1,776)	(1,616)	(590)
24	6,707.8	7,006.2	3,185.8
	(1,358)	(2,778)	(925)
48	7,316.6	6,035.4**	3,802.7***
	(2,049)	(958)	(1,703)
72	8,176.2	2,817.7**	5,005.6***
	(2,707)	(894)	(1,235)
96	5,181.3	2,776.5***	4,978.0**
	(4,703)	(913)	(3,458)
K_{uoc}	936.9	142.0	346.2
	(164)	(23)	(35)

TABLE A-III

BENZO(*a*)PYRENE ACCUMULATION IN *Chironomus riparius*

Mean Sediment Concentration: 0.42 ng/g dry weight (assay I; aged 1 week)
0.39 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	48.1	1.6***	19.1***	79.0***	3.2***	23.8***
	(24)	(1)	(15)	(50)	(1)	(2)
6	134.3*	11.2	18.0**	125.3*	27.0***	49.0**
	(53)	(7)	(4)	(49)	(3)	(20)
24	428.8	68.9	81.7**	504.3	234.4**	242.6***
	(146)	(24)	(5)	(150)	(137)	(54)
48	1,130.9	184.2	138.1**	1,130.5	353.4**	333.2**
	(325)	(76)	(19)	(436)	(186)	(96)
72	1,030.3*	207.5*	222.1**	1,410.1	697.2**	538.9**
	(273)	(98)	(53)	(439)	(359)	(123)
96	1,351.1	321.0	285.2***	1,308.1	329.2***	506.0**
	(668)	(70)	(153)	(195)	(151)	(171)
K_{uoc}	15.73	2.72	3.25	14.96	4.40	7.64
	(1.4)	(0.3)	(0.2)	(1.4)	(2.2)	(0.6)

TABLE A-III (Continued)

Compound: Benzo(*a*)pyrene
Species: *Chironomus riparius*
Mean Sediment Concentration: 0.41 ng/g dry weight (assay III; aged 2 months)
0.41 ng/g dry weight (assay IV; aged 6.5 months)

	Assay III			Assay IV		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	59.4*	57.5***	14.4**	---	---	---
	(26)	(51)	(3)			
6	72.6*	46.5***	68.6**	95.9**	106.8	33.7**
	(46)	(50)	(59)	(28)	(62)	(5)
24	---	362.8	364.7**	455.0*	352.2*	492.5**
		(141)	(121)	(145)	(139)	(265)
48	237.0	268.6	282.3**	672.4	461.8	726.3**
	(87)	(116)	(133)	(324)	(123)	(115)
72	353.1	577.3*	511.4**	965.8	732.6	766.5**
	(123)	(155)	(122)	(383)	(263	(159)
96	524.8	328.9	398.2**	1,280.4	686.9	818.5**
	(190)	(135)	(214)	(202)	(186)	(57)
K_{uoc}	23.49	1.20	11.18	12.57	6.64	0.86
	(3.0)	(0.2)	(0.6)	(1.4)	(1.0)	(0.1)

TABLE A-IV

ENDRIN ACCUMULATION IN *Chironomus riparius*

Mean Sediment Concentration: 1,477.4 ng/g dry weight (assay I; aged 1 week)
527.2 ng/g dry weight (assay II; aged 1 month)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	3,498.8	1,399.4**	1,232.8**	1,580.1	626.2**	1,555.9
	(760)	(298)	(363)	(493)	(60)	(482)
6	11,636.4	3,975.2**	2,847.2	4,515.3)	1,465.3**	2,370.5
	(3,510)	(186)	(873)	(1.209)	(236)	(433)
24	13,871.4	6,315.3	2,668.9	6,700.4	3,531.2	4,417.7
	(2,183)	(1,982)	(307)	(2,534)	(1,227)	(847)
48	13,143.7	6,602.9**	4,934.2**	11,327.7	5,581.1	8,124.7
	(5,448)	(1,448)	(1,150)	(1,915)	(2,090)	(1,657)
72	14,231.9	2,453.6**	3,143.6***	10,117.4*	5,598.2	10,469.6
	(3,487)	(235)	(806)	(2,658)	(1,646)	(904)
96	15,562.4**	1,910.8***	1,946.4	9,841.4	6,156.2	11,473.0
	(3,590)	(208)	(703)	(2,763)	(2,264)	(1,735)
K_{uoc}	1,726.9	511.4	298.3	189.2	113.8	128.2
	(308)	(47)	(93)	(21)	(12)	(9)

TABLE A-V

trans-CHLORDANE ACCUMULATION IN *Diporeia* SPP.

Mean Sediment Concentration: 493.1 ng/g dry weight (assay I; aged 1 week)
430.3 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	755.1 (252)	291.2 (121)	300.0 (69)	---	---	---
6	2,747.4 (449)	1,004.3 (172)	738.4 (177)	2,100.4 (455)	829.4 (310)	909.8 (148)
24	7,174.0 (1,328)	2,940.6* (1,059)	1,793.0 (295)	6,960.4 (2,768)	1,863.6** (499)	2,425.1 (1,082)
48	16,470.4 (1,963)	5,695.7* (1,663)	2,736.1 (842)	13,884.1* (4,773)	4,014.6 (808)	3,510.8** (518)
72	19,448.9 (6,456)	4,822.1 (1,313)	2,864.7 (570)	24,122.0* (15,939)	5,490.2 (1,749)	5,490.2** (1,749)
96	23,729.0 (5,566)	6,926.2 (1,269)	---	40,086.3* (7,827)	6,286.8 (2,423)	7,251.3 (3,327)
168	---	---	---	43,851.1** (11,875)	9,447.9 (2,273)	7,793.1** (1,291)
240	51,844.3* (12,965)	10,779.4* (1,877)	---	---	---	---
336	---	---	---	73,426.8 (15,790)	11,018.4 (2,363)	7,627.6** (1,370)
K_{uoc}	246.6 (18)	58.7 (4)	51.0 (4)	216.8 (13)	46.9 (3)	70.9 (4)

TABLE A-V (Continued)

Compound: *trans*-Chlordane
Species: *Diporeia* spp.
Mean Sediment Concentration: 318.7 ng/g dry weight (aged 1 week)

	Assay III		
Hours	Sediment	Elutriate	Porewater
1	324.9	335.9	296.4
	(133)	(32)	(58)
6	2,472.6	1,038.9	720.2*
	(641)	(99)	(155)
24	7,007.0	3,649.0	2,356.6
	(2,103)	(415)	(987)
48	12,680.2	5,313.2	4,926.2
	(2,059)	(578)	(1,533)
72	20,750.2*	7,676.5	3,585.8
	(2,651)	(1,567)	(779)
96	22,960.6*	7,314.3	5,586.5**
	(3,308)	(2,111)	(1,344)
168	38,955.8	6,168.7	4,771.4
	(8,379)	(2,658)	(878)
240	47,329.1	7,070.3	6,319.0
	(10,936)	(1,506)	(1,942)
336	58,797.4	6,611.3	5,124.6
	(25,161)	(1,204)	(533)
K_{uoc}	252.7	55.1	74.7
	(16)	(4)	(4)

TABLE A-VI

PYRENE ACCUMULATION IN *Diporeia* SPP.

Mean Sediment Concentration: 0.76 ng/g dry weight (assay I; aged 1 week)
0.73 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	619.5**	625.5**	619.1**	284.0*	512.7	---
	(97)	(161)	(143)	(41)	(93)	
6	2,929.3**	2,210.4**	1,782.2**	1,503.8	2,056.1	1,247.0**
	(409)	(82)	(698)	(399)	(502)	(241)
24	7,488.0**	4,776.2**	6,997.7**	5,150.8	5,117.1*	3,773.3**
	(2,118)	(1,222)	(2,249)	(951)	(2,496)	(629)
48	15,950.8**	11,518.9**	10,074.4**	8,584.4*	8,101.1	5,018.6**
	(3,714)	(5,265)	(3,439)	(1,697)	(1,678)	(1,284)
72	18,410.3**	12,784.4**	12,693.2**	9,246.0**	11,288.9	9,034.6**
	(4,424)	(4,577)	(2,039)	(2,817)	(2,126)	(684)
96	21,566.7**	19,603.8**	17,766.2**	12,512.2	13,834.4	10,301.8**
	(2,255)	(3,970)	(6,143)	(1,890)	(2,993)	(2,063)
168	29,075.6**	27,859.6**	15,646.5**	19,580.0	21,709.0	31,536.5**
	(3,935)	(3,900)	(6,683)	(3,708)	(7,047)	(4,184)
240	23,942.1**	17,307.1**	15,677.0**	23,521.7*	28,058.9	18,087.1**
	(5,967)	(4,200)	(7,256)	(4,638)	(8,707)	(2,262)
336	40,638.6**	31,524.0**	19,047.3**	35,508.5	32,247.1	---
	(9,353)	(6,647)	(6,068)	(8,356)	(8,295)	
K_{uoc}	319.1	134.1	250.0	176.0	158.2	133.3
	(28)	(13)	(22)	(12)	(14)	(10)

TABLE A-VII

BENZO(*a*)PYRENE ACCUMULATION IN *Diporeia* SPP.

Mean Sediment Concentration: 0.41 ng/g dry weight (assay I; aged 1 week)
0.27 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
6	266.2**	133.2	190.1**	219.8	105.1	111.0
	(96)	(37)	(32)	(56)	(34)	(14)
24	542.0	242.0**	365.3**	730.5*	184.3**	261.2
	(51)	(60)	(44)	(148)	(48)	(69)
48	583.2	546.5	581.2**	957.8*	492.0	412.2**
	(91)	(59)	(105)	(434)	(63)	(57)
72	1,186.9	836.8	892.6**	2,146.2**	699.6	520.3**
	(127)	(252)	(156)	(1,787)	(182)	(106)
96	1,740.6	1,129.1**	1,170.5***	3,562.7*	1,006.6	1,155.3
	(390)	(157)	(508)	(923)	(261)	(500)
168	3,597.4	1,739.7	1,753.1**	3,096.1	2,054.0	2,412.1
	(772)	(152)	(376)	(840)	(649)	(364)
240	3,214.3*	3,548.6*	2,282.6**	---	---	---
	(1,498)	(756)	(372)			
336	6,958.6	3,083.1*	3,028.4***	6,615.8	3,933.5	3,940.9**
	(550)	(1,177)	(1,347)	(2,257)	(709)	(1,351)
K_{uoc}	15.32	8.72	12.52	19.55	3.88	6.91
	(2.0)	(0.2)	(0.6)	(1.5)	(0.2)	(0.4)

TABLE A-VII (Continued)

Compound: Benzo(*a*)pyrene
Species: *Diporeia* spp.
Mean Sediment Concentration: 0.38 ng/g dry weight (aged 1 week)

	Assay III		
Hours	Sediment	Elutriate	Porewater
1	95.3*	27.7***	31.9**
	(26)	(24)	(22)
6	187.1	69.9	122.3
	(50)	(33)	(44)
24	404.6	178.6	400.1
	(139)	(32)	(80)
48	761.2*	397.2	704.1
	(199)	(128)	(140)
72	704.2	389.7	1,341.9
	(436)	(138)	(572)
96	1,146.4	590.0	---
	(635)	(144)	
168	2,497.0*	1,742.9*	---
	(732)	(401)	
K_{uoc}	11.00	4.26	14.19
	(1.4)	(0.2)	(1.0)

TABLE A-VIII

DDT ACCUMULATION IN *Diporeia* SPP.

Mean Sediment Concentration: 367.3 ng/g dry weight (aged 1 week)

	Assay I		
Hours	Sediment	Elutriate	Porewater
1	495.7	554.2	1,026.5*
	(257)	(187)	(300)
6	1,354.4*	1,306.3	1,945.2*
	(667)	(150)	(432)
24	2,796.9**	3,373.6	3,597.5
	(665)	(461)	(845)
48	4,841.0**	4,394.9	4,396.6
	(1,632)	(944)	(852)
72	7,187.6*	6,637.6*	5,611.3
	(1,553)	(1,694)	(947)
96	13,314.0*	7,563.5	8,346.2
	(6,336)	(1,044)	(1,292)
168	25,342.1*	9,167.4	11,620.1
	(12,862)	(1,036)	(1,022)
240	34,869.7	9,777.8	16,800.9*
	(9,472)	(1,269)	(2,279)
336	31,702.4*	16,072.2	18,890.1
	(5,386)	(5,538)	(1,570)
K_{uoc}	91.0	120.5	205.7
	(5)	(7)	(43)

TABLE A-IX

CHRYSENE ACCUMULATION IN *Diporeia* SPP.

Mean Sediment Concentration: 34.4 ng/g dry weight (aged 1 week)

	Assay I		
Hours	Sediment	Elutriate	Porewater
1	765.0	1,579.9	865.6*
	(182)	(552)	(252)
6	1,450.4	2,102.4	1,972.4
	(397)	(722)	(763)
24	3,732.6*	3,616.8	4,455.4
	(145)	(1,339)	(1,192)
48	5,737.6**	5,148.6	5,514.5
	(1,680)	(991)	(1,024)
72	8,341.3*	7,297.6*	7,020.0
	(1,715)	(1,629)	(1,054)
96	14,046.9	8,207.6	10,089.4
	(4,520)	(451)	(1,690)
168	18,565.3*	9,777.0	12,883.8
	(1,959)	(1,646)	(1,819)
240	29,249.8	11,035.6	16,371.6*
	(5,424)	(1,709)	(2,687)
336	26,823.9*	12,803.4	15,661.1
	(3,418)	(1,632)	(3,287)
K_{uoc}	128.5	55.2	244.3
	(7)	(13)	(33)

TABLE A-X

trans-CHLORDANE ACCUMULATION IN *Lumbriculus variegatus*

Mean Sediment Concentration: 124.6 ng/g dry weight (assay I; aged 1 week)
1,405.9 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	978.4***	401.1	266.0	1,372.3	312.4	392.0
	(339)	(112)	(28)	(283)	(69)	(96)
6	2,763.4	1,069.7	498.2	1,966.3	827.0	595.0
	(516)	(128)	(106)	(607)	(284)	(97)
24	9,065.5	3,565.1	1,530.2	9,211.7	2,291.8	1,594.0
	(2,646)	(222)	(348)	(1,843)	(410)	(265)
48	13,649.8	5,682.3	2,674.2	12,903.7*	3,372.1	2,366.6*
	(4,610)	(929)	(414)	(3,616)	(652)	(376)
72	18,141.3	8,854.4	3,718.3	22,954.4**	3,645.9**	1,929.0**
	(5,133)	(530)	(499)	(1,671)	(618)	(222)
96	28,197.4	11,604.4	5,668.9	23,030.8	5,671.8*	---
	(11,553)	(2,127)	(900)	(2,981)	(1,074)	
168	42,264.1	17,321.3	8,066.1	34,150.0	5,609.5**	5,007.4***
	(6,231)	(4,217)	(2,120)	(6,063)	(379)	(365)
K_{uoc}	355.6	117.9	57.5	356.6	72.8	63.3
	(42)	(4)	(4)	(29)	(5)	(4)

TABLE A-XI

PYRENE ACCUMULATION IN *Lumbriculus variegatus*

Mean Sediment Concentration: 0.65 ng/g dry weight (assay I; aged 1 week)
0.14 ng/g dry weight (assay II; aged 1 week)

	Assay I			Assay II		
Hours	Sediment	Elutriate	Porewater	Sediment	Elutriate	Porewater
1	390.6	258.4	343.6	597.9**	556.7	255.9
	(129)	(84)	(61)	(247)	(231)	(41)
6	918.6	1,136.5	845.4	1,575.8	1,150.6	491.4
	(391)	(184)	(221)	(331)	(250)	(210)
24	4,523.4	3,475.4	2,361.2	4,174.3	4,183.0	1,484.8
	(808)	(566)	(338)	(1,472)	(478)	(176)
48	5,495.8**	5,562.8	2,889.2*	5,869.1	5,250.4	2,384.9
	(1,091)	(1,295)	(463)	(1,206)	(579)	(454)
72	7,681.6**	6,915.0**	3,264.0**	5,565.0	8,071.2	2,383.6
	(1,080)	(1,523)	(729)	(1,582)	(975)	(249)
96	7,303.6	8,763.1*	---	7,688.5	11,694.0	4,390.6
	(491)	(1,127)		(1,442)	(2,196)	(596)
168	8,302.8	11,930.6**	5,383.0***	5,856.6	18,548.8	4,657.4
	(1,564)	(1,092)	(212)	(594)	(4,489)	(1,141)
K_{uoc}	185.2	95.5	90.7	153.2	82.0	55.0
	(13)	(5)	(5)	(23)	(5)q	(3)

TABLE A-XII

BENZO(*a*)PYRENE ACCUMULATION IN *Lumbriculus variegatus*

Mean Sediment Concentration: 80.9 ng/g dry weight (aged 1 week)

	Assay I		
Hours	Sediment	Elutriate	Porewater
1	88.8	79.5	40.5
	(62)	(66)	(20)
6	285.3	46.2	195.3
	(163)	(27)	(44)
24	506.8	264.2*	608.5
	(173)	(163)	(144)
48	1,510.0	609.7	1,375.0
	(852)	(173)	(96)
72	3,811.3	1,381.5	2,477.9
	(1,284)	(658)	(1,187)
96	4,496.2	1,599.1	3,310.1
	(932)	(590)	(514)
168	5,356.5	2,411.9	4,718.6
	(539)	(904)	(975)
K_{uoc}	53.5	8.1	30.4
	(5)	(0.7)	(2.0)

Appendix B

Lipid Content in Indicator Species

TABLE B-I

TOTAL LIPID CONTENT OF INDICATOR SPECIES AFTER VARIOUS EXPOSURES IN WHOLE SEDIMENT, POREWATER, ELUTRIATE, AND DOSED LAKE MICHIGAN WATER

Organism	Exposure Temperature (°C)	Days Exposed	Media	Compound	Percent Lipid[1]	n
C. riparius	23	---	culture water	undosed	3.72 (2.0)[2]	9
C. riparius	10	3	sediment	undosed	8.31 (7.2)	14
C. riparius	10	3	porewater	*t*-chlordane & BaP	5.11	1
C. riparius	10	3	lake water	*t*-chlordane & BaP	3.44 (2.2)	3
C. riparius	10	3	sediment	*t*-chlordane & BaP	3.86 (1.7)	5
C. riparius	10	3	porewater	pyrene	5.86	2
C. riparius	10	3	elutriate	pyrene	5.50 (4.3)	3
C. riparius	10	3	lake water	pyrene	3.7	1

TABLE B-I (Continued)

Organism	Exposure Temperature (°C)	Days Exposed	Media	Compound	Percent Lipid	n
C. riparius	10	3	sediment	pyrene	1.25 (1.0)	3
C. riparius	10	3	sediment	pyrene	5.56 (4.2)	14
Diporeia spp.	10	---	sediment	undosed	33.91 (9.4)	6
Diporeia spp.	10	14	sediment	*t*-chlordane & BaP	24.82 (2.7)	6
Diporeia spp.	10	14	lake water	pyrene	36.30 (3.4)	7
Diporeia spp.	10	14	sediment	pyrene	33.51 (7.5)	8
L. variegatus	23	---	culture water	undosed	13.17 (4.3)	8
L. variegatus	23	7	sediment	undosed	4.92 (1.7)	22
L. variegatus	23	---	culture water	undosed	7.01 (1.9)	9
L. variegatus	10	7	porewater	*t*-chlordane & pyrene	5.27 (2.8)	3

TABLE B-I (Continued)

Organism	Exposure Temperature (°C)	Days Exposed	Media	Compound	Percent Lipid	n
L. variegatus	10	3	sediment	*t*-chlordane & pyrene	8.78 (2.4)	3
L. variegatus	10	3	sediment	*t*-chlordane & pyrene	5.42 (1.6)	

[1]Lipid is percent of total dry weight

[2]$\pm$ 1 standard deviation

Appendix C

Biotransformation Studies

TABLE C-I

PERCENTAGE OF PARENT COMPOUNDS IN INDICATOR SPECIES AFTER VARIOUS EXPOSURES IN WHOLE SEDIMENT AND DOSED LAKE MICHIGAN WATER, AS ASSAYED VIA THIN LAYER CHROMATOGRAPHY AND LIQUID SCINTILLATION TECHNIQUES

Organism	Exposure Temperature (°C)	Days Exposed	Media	Compound	Percent Parent Compound
C. riparius	23	2	lake water	BaP	25.9[1]
C. riparius	10	4	sediment	BaP	3.1
C. riparius	10	4	sediment	*t*-chlordane	100.0
C. riparius	10	4	sediment	pyrene	26.4
C riparius	10	4	sediment	pyrene	28.5
Diporeia	10	4	lake water	BaP	92.7
Diporeia	10	4	lake water	*t*-chlordane	95.1
Diporeia	10	4	lake water	*t*-chlordane	91.6
Diporeia	10	14	sediment	pyrene	98.8
Diporeia	10	14	lakewater	pyrene	99.5
Diporeia	10	4	lake water	pyrene	94.6
L. variegatus	10	8	sediment	BaP	97.6
L. variegatus	10	8	lake water	BaP	97.8
L. variegatus	10	7	sediment	BaP	92.2
L. variegatus	10	4	sediment	BaP	94.2
L. variegatus	10	4	sediment	*t*-chlordane	92.7
L. variegatus	10	4	sediment	pyrene	94.2

[1]Values represent extracts of 10-20 individuals.

LITERATURE CITED

Adams, W. J. 1987. Bioavailability of neutral lipophilic organic chemicals contained on sediments: A review. In: *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems.* Proceedings of the Sixth Pellston Workshop, Florissant, CO, August 12, 1984. K. L. Dickson, A. W. Maki, and W. A. Brungs, eds. Pergamon Press, Elmsford, NY. pp. 219-244.

Adams, W. J., R. A. Kimerle, and R. G. Mosher. 1984. Aquatic safety assessment of chemicals sorbed to sediments. In: *Aquatic Toxicology and Hazard Assessment: Seventh Symposium.* ASTM STP 854. R. D. Cardwell, R. Purdy, and R. C. Bahner, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 429-453.

Adams, W. J., P. S. Ziegenfuss, W. J. Renaudette, and R. G. Mosher. 1986. Comparison of laboratory and field methods for testing the toxicity of chemicals sorbed to sediments. In: *Aquatic Toxicology and Environmental Fate: Ninth Volume.* ASTM STP 921. T. M. Poston and R. Purdy, eds. American Society for Testing and Materials, Philadelphia. PA. pp. 494-513.

Amato, J. R., D. I. Mount, E. J. Durhan, M. T. Lukasewycz, G. T. Ankley, and E. D. Robert. 1992. An example of the identification of diazinon as a primary toxicant in an effluent. Environ. Toxicol. Chem. 11:209-216.

Anderson, J., W. Birge, J. Gentile, J. Lake, J. Rodgers, Jr., and R. Swartz. 1984. Biological effects, bioaccumulation, and ecotoxocology of sediment-associated chemicals In: *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems.* K. L. Dickson, A. W. Maki, and W. A. Brungs, eds., Pergamon Press, Elmsford, NY. pp. 267-296.

Ankar, S. 1977. The soft bottom ecosystem of the Northern Baltic Proper with special reference to the macrofauna. *Contrib. Asko. Lab. Univ. Stockholm.* 19:1-62.

Ankley, G. T., M. K. Schubauer-Berigan, and J. R. Dierkes. 1991. Predicting the toxicity of bulk sediments to aquatic organisms with aqueous test fractions: Pore water vs. elutriate. Environ. Toxicol. Chem. 10:1359-1366.

Ankley, G. T., D. A. Benoit, R. A. Hoke, E. N. Leonard, C. W. West, G. L. Phipps, V. R. Mattson, and L. A. Anderson. 1993*a*. Development and evaluation of test methods for benthic invertebrates and sediments: Effects of flow rate and feeding on water quality and exposure conditions. Arch. Environ. Contam. Toxicol. 25:12-19.

Ankley, G. T., D. A. Benoit, J. C. Balogh, T. B. Reynoldson, K. E. Day, and R. A. Hoke. 1993*b*. Evaluation of potential confounding factors in sediment toxicity tests with three freshwater invertebrates. Environ. Toxicol. Chem. In press.

ASTM. 1992*a*. Standard guide for conducting sediment toxicity tests with freshwater invertebrates. Designation E-1383-92. *Annual Book of ASTM Standards, Vol 11.04.* American Society for Testing and Materials, Philadelphia, PA. pp. 1116-1138.

ASTM. 1992*b*. Standard guide for conducting 10-day static sediment toxicity tests with marine and estuarine amphipods. Designation E 1367-90. *Annual Book of ASTM Standards, Vol 11.04*. American Society for Testing and Materials, Philadelphia, PA. pp.1083-1106.

ASTM 1992*c*. Standard guide for collection, storage, characterization, and manipulation of sediments for toxicological testing. Designation E-1391-90. *Annual Book of ASTM Standards, Vol 11.04*. American Society for Testing and Materials, Philadelphia, PA. pp. 1139-1153.

Athey, L. A., J. M. Thomas, W. E. Miller, and J. Q. Word. 1989. Evaluation of bioassays for designing sediment cleanup strategies at a wood treatment site. Environ. Toxicol. Chem. 8:223-230.

Bahnick, D. A., W. A. Swenson, T. P. Markee, D. J. Call, C. A. Anderson, and R. T. Morris. 1981. Development of bioassay procedures for defining pollution of harbor sediments. Project Summary EPA-600/S3-81-025. Environmental Research Laboraotry, Duluth, MN.

Bailey, H. C. and D. H. W. Liu. 1980. *Lumbriculus variegatus*, a benthic oligochaete, as a bioassay organism. In: *Aquatic Toxicology*, ASTM STP 707. J. C. Eaton, P. R. Parrish, and A. C. Hendricks, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 205-215.

Barnes, R. O. 1973. An *in situ* interstitial water sampler for use in unconsolidated sediments. Deep Sea Res. 20:1125-1128.

Barron, M. G., G. R. Stehly, and W. L. Hayton. 1990. Pharmacokinetics modeling in aquatic animals I. Models and concepts. Aquatic Tox. 17:187-212.

Becker, D. S., G. R. Bilyard, and T. C. Ginn. 1990. Comparisons between sediment bioassays and alterations of benthic macroinvertebrate assemblages at a marine superfund site: Commencement Bay, Washington. Environ. Toxicol. Chem. 9:669-685.

Bender, M., W. Martin, J. Hess, F. Sayled, L. Ball, and C. Lambert. 1987. A whole-ore squeezer for interfacial pore-water sampling. Limnol. Oceanogr. 32:1214-1225.

Birge, W. J., J. A. Black, A. G. Westerman, and P. C. Francis. 1984. Toxicity of sediment-associated metals to freshwater organisms: Biomonitoring p rocedures. In: *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*. K. L. Dickson, A. W. Maki, and W. A. Brungs, eds. Pergamon Press, Elmsford, NY. pp. 199-218.

Black, M. C. and J. F. McCarthy. 1988. Dissolved organic matter reduces the uptake of hydrophobic organic contaminants by gills of the rainbow trout, *Salmo gairdneri*. Environ. Toxcol. Chem. 7:593-600.

Boese, B. L., H. Lee II, D. T. Specht, R. C. Randall, and M. Winsor. 1990. Comparison of aqueous and solid-phase uptake for hexachlorobenzene in the tellinid clam, *Macoma nasuta* (Conrad): A mass balance approach. Environ. Toxicol. Chem. 9:221-231.

Bottomley, E. Z. and I. L. Bayly. 1984. A sediment porewater sampler used in root zone studies of the submerged macrophyte, *Myriophyllum spicatum*. Limnol. Oceanogr. 29:671-673.

Bousefield, E. L. 1989. Revised morphological relationships within the amphipod genera *Pontoporeia* and *Gammaracanthus* and the glacial relict significance of their postglacial distributions. *Can. J. Fish. Aquat. Sci.* 46:1714-1725.

Boyd, M. B., P. T. Saucier, J. W. Keeley, R. L. Montgomery, R. D. Brown, D. B. Mathis, and C. J. Guice. 1972. Disposal of dredge spoil: Problems identification and assessment and research program development. Technical Report H-72-8. U. S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

Brannon, J. M., R. H. Plumb, Jr., and I. Smith, Jr. 1980. Long-term release of heavy metals for sediments. In: *Contaminants and Sediments, Volume I.* R. A. Baker, ed. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 221-266.

Brinkman, A. G., W. van Raaphorst, and L. Lijklema. 1982. *In situ* sampling of interstitial water from lake sediments. Hydrobiologia 92:659-663.

Brouwer, H., T. Murphy, and L. McArdle. 1990. A sediment-contact bioassay with *Photobacterium phosphoreum.* Environ. Toxicol. Chem. 9:1353-1358.

Burres, R. M. and J. H. Chandler. 1976. Use of the Asiatic clam, *Corbicula leana* Prime in toxicity tests. Prog. Fish. Cult. 38:10.

Burton, G. A., Jr. 1991. Assessing the toxicity of freshwater sediments. Environ. Toxicol. Chem. 10:1585-1627.

Burton, G. A., Jr., L. Burnett, M. Henry, S. Klaine, P. Landrum, and M. Swift. 1990. A multi-assay comparison of sediment toxicity at three "Areas of Concern." Abstracts, 11th Annual Meeting, Society of Environmental Toxicology and Chemistry, Arlington, VA., November 11-15, 1990. p.53.

Bysshe, S. E. 1982. Bioconcentration factor in aquatic organisms. In:*Handbook of Chemical Property Estimation Methods., Chapter 6.*. W. J. Lyman, W. F. Reehl, and D. H. Rosenblatt, eds. McGraw-Hill, New York, NY. pp. 5-1 to 5-30.

Cairns, M. A., A. V. Nebeker, J. H. Gakstatter, and W. L. Griffis. 1984. Toxicity of copper-spiked sediments to freshwater invertebrates. Environ. Toxicol. Chem. 3:435-445.

Cammen, L. 1989. The relationship between ingestion rate of deposit feeders and sediment nutritional value. In G. Lopez, G. Taghon, and J. Levinton, eds., *Lecture Notes on Coastal and Estuarine Studies: Ecology of Marine Deposit Feeders.* Springer-Verlag Publishers, New York, NY. pp. 201-222.

Capel, P. D. and S. J. Eisenreich. 1990. Relationship between chlorinated hydrocarbons and organic carbon in sediment and porewater. J. Great Lakes Res. 16:245-257.

Carignan, R. 1984. Interstitial water sampling by dialysis: Methodological notes. Limnol. Oceanogr. 29:667-670.

Carignan, R., F. Rapin, and A. Tessier. 1985. Sediment porewater sampling for metal analysis: A comparison of techniques. Geochimica et Cosmochimica Acta 49:2493-2497.

Carr, R. S., J. W. Williams, and C. T. B. Fragata. 1989. Development and evaluation of novel marine sediment pore water toxicity test with the polychaete *Dinophilus gyrociliatus.* Environ. Toxicol. Chem. 8:533-543.

Carter, C. W. and I. H. Suffet. 1982. Binding of DDT to dissolved humic materials. Environ. Sci. Technol. 16:735-740.

Chapman, P. M. 1988. Marine sediment toxicity tests. In: *Symposium on Chemical and Biological Characterization of Sludges, Sediments, Dredge Spoils and Drilling Muds*, ASTM STP 976. J. J. Lichenberg, F. A. Winter, C. I. Weber, and L. Fradkin, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 391-402.

Chapman, P. M. and R. Fink. 1984. Effects of Puget Sound sediments and their elutriates on the life cycle of *Capitella capitata*. Bull. Environ. Contam. Toxicol. 33:451-459.

Chew, R. D. and M. A. Hamilton. 1985. Toxicity curve estimation: Fitting a compartment model to median survival times. Trans. Amer. Fish. Soc. 114:403-412.

Chin, Y. and P. M. Gschwend. 1992. Partitioning of polycyclic aromatic hydrocarbons to marine porewater organic colloids. Environ. Sci. Technol. 26:1621-1626.

Chiou, C. T., V. H. Freed, D. W. Schmedding, and R. L. Kohnert. 1977. Partition coefficient and bioaccumulation of selected organic chemicals. Environ. Sci. Technol. 475-478.

Chiou, C. T., R. L. Malcolm, T. I. Brinton, and D. E. Kile. 1986. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. Environ. Sci. Technol. 20:502-508.

Coats, J. T. and A. W. Elzerman. 1986. Desorption kinetics for selected PCB congeners from river sediments. J. Contamin. Hydrol. 1:191-210.

Coffman, W. P. 1967. *Community structure and tropic relations in a small woodland stream, Linesville Creek, Crawford County, Pennsylvania*. Ph.D. thesis, Univ. Pittsburgh, Pittsburgh, PA.

Connolly, J. P. 1985. Predicting single-species toxicity in natural water systems. Environ. Toxicol. Chem. 4:573-582.

Conway, R. A. 1982. *Environmental Risk Analysis for Chemicals*. Van Nostrand Reinhold Company, New York, NY.

Daniels, S. A., M. Munawar, and C. I. Mayfield. 1989. An improved elutriation technique for the bioassessment of sediment contaminants. Hydrobiologia 188/189:619-631.

Data Description, Inc. 1985. *Data Desk® Professional User's Guide, Version 2.0*. Odesta Corp., Northbrook, IL.

DiToro, D. M., L. M. Horzempa, M. M. Casey and W. Richardson. 1982. Reversible and resistant components of PCB adsorption-desorption: Adsorption concentration effects. J. Great Lakes Res. 8:336-349.

DiToro, D. M., J. D. Mahony, D. J. Hansen, K. J. Scott, M. B. Hicks, S. M. Mayr, and M. S. Redmond. 1990. Toxicity of cadmium in sediments: The role of acid volatile sulfide. Environ. Toxicol. Chem. 9:1487-1502.

DiToro, D. M., C. S. Zarba, D. J. Hansen, W. J. Berry, R. C. Swartz, C. E. Cowan, S. P. Pavlou, H. E. Allen, N. A. Thomas, and P. R. Paquin. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ. Toxicol. Chem. 10:1541-1583.

Dutka, B. J. and K. K. Kwan. 1988. Battery of screening tests approach applied to sediment extracts. Toxicity Assessment: An International Journal 3:303-314.

Eadie, B. J., W. R. Faust, P. F. Landrum, and N. R. Morehead. 1985. Factors affecting bioconcentration of PAH by the dominant benthic organisms of the Great Lakes. In: *Polynuclear Aromatic Hydrocarbons: Eighth International Symposium on Mechanisms, Methods and Metabolism.* M. W. Cooke and A. J. Dennis, eds. Battelle Press, Columbus, OH. pp. 363-377.

Eadie, B. J., N. R. Morehead, and P. F. Landrum. 1990. Three-phase partitioning of hydrophobic organic compounds in Great Lakes waters. Chemosphere 20:161-178.

Eadie, B. J., N. R. Morehead, J. V. Klump, and P. F. Landrum. 1992. Distribution of hydrophobic organic compounds between dissolved and particulate organic matter in Green Bay waters. J. Great Lakes Res. 18:91-97.

Edmunds, W. M., and A. H. Bath. 1976. Centrifuge extraction and chemical analysis of interstitial waters. Environ. Sci. Technol. 10:467-492.

Engler, R. M. 1980. Prediction of pollution potential through geochemical and biological procedures: Development of regulation and criteria for the discharge of dredged and fill material. In: *Contaminants and Sediments, Volume I.* R. A. Baker, ed. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 143-170.

Erickson, C. 1968. Ecological significance of respiration and substrate for burrowing Ephemeroptera. Can. J. Zool. 46:93-103.

Ernst, W. 1977. Determination of the bioconcentration potential of marine organisms - A steady state approach. Chemosphere 11:731-740.

Evans, M. S. and P. F. Landrum. 1989. Toxicokinetics of DDE, benzo(*a*)pyrene, and 2,4,5,2',4',5'-hexachlorobiphenyl in *Pontoporeia hoyi* and *Mysis relicta.* J. Great Lakes Res. 15:589-600.

Ferraro, S. P., H. Lee II, R. J. Ozretich, and D. T. Specht. 1990. Predicting bioaccumulation potential: A test of a fugacity based model. Arch. Environ. Contamin. Toxicol. 19:386-394.

Finney, D. J. 1978. *Statistical Methods in Biological Assay, 3rd edition.* C. Griffin and Co. Ltd., London, England.

Fisher, S. W. 1985. Effects of pH on the toxicity and uptake of [^{14}C] lindane in the midge, *Chironomus riparius.* Ecotox. Environ. Safety 10:202-208.

Fisher, S. W. 1986. Effects of temperature on the acute toxicity of PCP in the midge *Chironomus riparius* Meigen. Bull. Environ. Contam. Toxicol. 36:744-748.

Fisher, S. W. and R. W. Wadleigh. 1986. Effects of pH on the acute toxicity and uptake of [^{14}C] pentachlorophenol in the midge, *Chironomus riparius.* Ecotox. Environ. Safety 11:1-8.

Forstner, U. 1990. Inorganic sediment chemistry and elemental speciation. In: *Chemistry and Toxicity of In-place Pollutants.* R. Baudo, J. Giesy, and H. Muntau, eds. Lewis Publishers, Boca Raton, FL. pp. 61-106.

Francis, P. C., W. J. Birge, and J. A. Black. 1984. Effects of cadmium-enriched sediment on fish and amphibian embryo-larval stages. Ecotoxocol. Environ. Saf. 8:378-387.

Fry, D. M. and S. W. Fisher. 1990. Effect of sediment contact and uptake mechanisms on accumulation of three chlorinated hydrocarbons in the midge, *Chironomus riparius.* Bull. Environ. Contamin. Toxicol. 44:790-797.

Gardner, W. S. T. F. Nalepa, W. A. Frez, E. A. Cichocki, and P. F. Landrum. 1985*a*. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. Can J. Fish. Aquat. Sci. 42:1827-1832.

Gardner, W. S., W. A. Frez, and E. A. Cichocki. 1985*b*. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. 30:1099-1105.

Gerking, S. D., A. Berg, and P. Gerard. 1976. Rate of sediment intake by midge larvae (*Chironomus plumosus*: Diptera) using a ^{134}Cs tracer; In: *Radioecology and Energy Resources. The Ecological Society of America Special Publication #1.*C. E. Cushing, Jr., ed. Dowden, Hutchinson, and Ross, Inc., Stroudsburg, PA, pp. 126-135.

Gerould, S., P. Landrum, and J. P. Giesy. 1983. Anthracene bioconcentration and biotransforation in chironomids: Effects of temperature and concentration. Environ. Poll. (Series A) 30:175-188.

Geyer, H., P. Sheehan, D. Kotzias, D. Freitag, and F. Korte. 1982. Prediction of ecotoxicological behavior of chemicals: Relationship between physio-chemical properties and bioaccumulation of organic chemicals in the mussel *Mytilus edulis.* Chemosphere 11:1121.

Giesy, J. P. and R. A. Hoke. 1989. Freshwater sediment toxicity bioassessment: Rationale for species selection and test design. J. Great Lakes Res. 15:539-569.

Giesy, J. P., R. L. Graney, J. L. Newsted, C. J. Rosiu, A. Benda, R. G. Kreis, Jr., and F. J. Horvath. 1988. Comparison of three sediment bioassay methods using Detroit River sediments. Environ. Toxicol. Chem. 7:483-498.

Giesy, J. P., C. J. Rosiu, and R. L. Graney. 1990. Benthic invertebrate bioassays with toxic sediment and pore water. Environ. Toxicol. Chem. 9:233-248.

Gobas, F. A. P. C. and D. Mackay. 1987. Dynamics of hydrophobic organic chemical bioconcentration in fish. Environ. Toxicol. Chem. 6:495-504.

Gobas, F. A. P. C., D C. G. Muir, and D. Mackay. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish Chemosphere 17:943-962.

Gobas, F. A. P. C., D. C. Bedard, J. J. H. Ciborowski, and G. D. Haffner. 1989*a*. Bioaccumulation of chlorinated hydrocarbons by the mayfly (*Hexagenia limbata*) in Lake St. Clair. J. Great Lakes Res. 15:581-588.

Gobas, F. A. P. C., K. E. Clark, W. Y. Shiu, and D. Mackay. 1989*b*. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: Role of bioavailability and elimination into the feces. Environ. Toxicol. Chem. 8:231-245.

Golterman, H. L., R. S. Clymo, and M. A. M. Ohnstad. 1978. *Methods for Physical and Chemical Analysis of Freshwaters*. IBP Handbook 8. Blackwell Publishers, Oxford, England.

Graney, R. L. and J. P. Giesy. 1988. Alterations in the oxygen consumption, condition index and concentration of free amino acids in *Corbicula fluminea* (Mollusca: Pelecypoda) exposed to sodium dodecyl sulfate. Environ. Toxicol. Chem. 7:310-315.

Green, A. S., G. T. Chandler, and E. R. Blood. 1993. Aqueous-, pore-water-, and sediment-phase cadmium: Toxicity relationships for a meiobenthic copepod. Environ. Toxicol. Chem. 12:1497-1506.

Hall, W. S., K. L. Dickson, F. Y. Saleh, and J. H. Rodgers, Jr. 1986. Effects of suspended solids on the bioavailability of chlordane to *Daphnia magna*. Arch. Environ. Contamin. Toxicol. 15:529-534.

Halter, M. T. and Johnson, H. E. 1977. A model system to study the desorption and biological availability of PCB in hydrosoils. In: *Aquatic Toxicology and Hazard Evaluation*, ASTM STP 634. F. L. Mayre and J. L. Hamelink, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 178-195.

Harkey, G. A., M. J. Lydy, J. Kukkonen, and P. F. Landrum. 1993*a*. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. Environ. Toxicol. Chem. In review.

Harkey, G. A., P. F. Landrum, and S. J. Klaine. 1993*b*. Partition coefficients of hydrophobic contaminants in natural water, porewater, and elutriates obtained from dosed sediment: A comparison of methodologies. Chemosphere. In press.

Hayes, W. J. Jr. and E. R. Laws, Jr. 1991. *Handbook of Pesticide Toxicology, Vol. I: Classes of Pesticides*. Academic Press, Inc., New York, NY.

Henry, M. G., D. N. Chester, and W. L. Mauck. 1986. Role of artificial burrows in *Hexagenia* toxicity tests: Recommendations for protocol development. Environ. Toxicol. Chem. 5:553-559.

Hesslein R. H. 1976. An *in situ* sampler for close interval pore water studies. Limnol. Oceanogr. 21:912-914.

Hoepner, T. 1981. Design and use of a diffusion sampler for interstitial water from fine grained sediments. Environmental Technology Letters 2:187-196.

Hoke, R. A., J. P. Giesy, G. T. Ankley, J. L. Newsted, and J. R. Adams. 1990. Toxicity of sediments from Western Lake Erie and the Maumee River at Toledo, Ohio: Implications for current dredged material disposal practices. J. Great Lakes Res. 16:457-470.

Howes, B. L. and S. G. Wakeham. 1985. Effects of sampling technique on measurements of porewater constituents in salt marsh sediments. Limnol. Oceanogr. 30:221-227.

Ingersoll, C. G. and M. K. Nelson. 1990. Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera). In: *Aquatic Toxicology and Risk Assessment: 13th Volume.* ASTM STP 1096. W. Landis and W. H. Van der Schalie, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 93-109.

Ivlev, V. S. 1939. Balance of energy of carps. Zool. Z. 18:303-318.

Ivlev, V. S. 1939. Transformation of energy by aquatic animals: Coefficient of energy consumption by *Tubifex tubifex* (Oligochaeta). Int. Rev. Hydrobiol. and Hydrogr. 38:449-458.

James, M. O. 1989. Biotransformation and disposition of PAH in aquatic invertebrates. In: *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* U. Varanasi, ed. CRC Press, Boca Raton, FL. 341 pp.

Jones, R. A., and G. F. Lee. 1978. Evaluation of the elutriate test as a method of predicting contaminant release during open water disposal of dredged sediment and environmental impact of open water dredged material disposal, Vol I - Discussion, Technical Report D-78-45. U. S. Army Engineer, Waterways Experiment Station, Vicksburg, MS.

Karickhoff, S. W. 1980. Sorption kinetics of hydrophobic pollutants in natural sediments. In: *Contaminants and Sediments,* Vol. 2. R. A. Baker, ed. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 193-206.

Karickhoff, S. W. and K. R. Morris. 1985. Impact of tubificid oligochaetes on pollutant transport in bottom sediments. Environ. Sci. Technol. 19:51-56.

Keilty, T. J. and P. F. Landrum. 1990. Population-specific toxicity responses by the freshwater oligochaete, *Stylodrilus heringianus,* in natural Lake Michigan sediments. Environ. Toxicol. Chem. 9:1147-1154.

Kemp, P. F. and R. C. Swartz. 1988. Acute toxicity of interstitial and particle-bound cadmium to a marine infaunal amphipod. Mar. Environ. Res. 26:135-153.

Kenaga, E. E. and C. A. I. Goring. 1980. Relationship between water solubility, soil sorption, octanol-water partitioning, and bioconcentration of chemicals in biota. In: *Third Aquatic Toxicology Symposium.* ASTM STP 707. J. G. Eaton, P. R. Parrish, and A. C. Hendricks, eds. American Society for Testing and Materials, Philadelphia, PA.

Klaassen, C. 1985. Nonmetallic environmental toxicants: Air pollutants, solvents and vapors, and pesticides. In: *The Pharmacological Basis of Therapeutics.* A. G. Gilman, L. S. Goodman, T. W. Rall, and F. Murad, eds. Macmillan Publishing Co., New York, NY. p. 1641.

Klopffer, W., G. Kaufmann, G. Rippen and H. J. Poremski. 1982. A laboratory method for testing the volatility from aqueous solution: First results and comparison with theory. Ecotoxicol. Environ. Safety 6:545-559.

Klump, J. V., J. R. Krezoski, M. E. Smith, and J. L. Kaster. 1987. Dual tracer studies of the assimilation of an organic contaminant from sediments by deposit feeding oligochaetes. Can. J. Fish. Aquat. Sci. 44:1574-1583.

Klump, J. V., J. L. Kaster, and M. E. Sierszen. 1991. Mysis relicta assimilation for hexachlorobiphenyl for sediments. Can. J. Fish. Aquat. Sci. 48:284-289.

Knezovich, J. P. and F. L. Harrison. 1988. The bioavailability of sediment-sorbed chlorobenzenes to larvae of the midge, *Chironomus decorus*. Ecotox. Environ. Safety 15:226-241.

Knezovich, J. P., F. L. Harrison, and R. G. Wilhelm. 1987. The bioavailability of sediment-sorbed organic chemicals: A review. Water, Air, and Soil Pollut. 32:233-245.

Kukkonen, J. 1991. *Effects of Dissolved Organic Material in Fresh Waters on the Binding and Bioavailability of Organic Pollutants*. Ph.D thesis, Univ. Joensuu, Joensuu, Finland.

Kukkonen, J. and A. Oikari. 1991. Bioavailability of organic pollutants in boreal waters with varying levels of dissolved organic material. Wat. Res. 25:455-463.

Kukkonen, J., J. F. McCarthy, and A. Oikari. 1990. Effects of XAD-8 fractions of dissolved organic carbon on the sorption and bioavailability of organic micropollutants. Arch. Environ. Contam. Toxicol. 19:551-557.

Lake, J. L., N. Rubenstein, and S. Pavignano. 1984. Predicting bioaccumulation: Development of a simple partitioning model for use as a screening tool for regulating ocean disposal of wastes. In: *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*. K. L. Dickson, A. W. Maki, and W. A. Brungs, eds. Pergamon Press, New York, NY. pp.151-166.

Landrum, P. F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediments for the amphipod *Pontoporeia hoyi*. Environ. Sci. Technol. 23:588-595.

Landrum, P. F. and J. P. Giesy. 1981. Anomalous breakthrough of benzo[a]pyrene during concentration with Amberlite® XAD-4 resin from aqueous solutions. In: *Advances in the Identification & Analysis of Organic Pollutants in Water, Volume 1*. L. H. Keith, ed. Ann Arbor Science Publishers, Ann Arbor, MI. pp. 345-355.

Landrum, P. F. and J. A. Robbins. 1990. Bioavailability of sediment-associated contaminants to benthic invertebrates. In: *Sediments: Chemistry and Toxicity of In-Place Pollutants*. R. Baudo, J. P. Giesy, and H. Muntau, eds. Lewis Publishers, Ann Arbor, MI. pp. 237-263.

Landrum, P. F. and W. R. Faust. 1991. Effect of variation in sediment composition on the uptake rate coefficient for selected PCB and PAH congeners by the amphipod *Diporeia* spp. In: *Aquatic Toxicology and Risk Assessment: Fourteenth Volume*. ASTM STP 1124. M. A. Mayes and M. G. Barron, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 263-279.

Landrum, P. F. and M. J. Lydy. 1991. Toxicokinetics Short Course. Presented at the 12th Annual Meeting, Society of Environmental Toxicology and Chemistry, November 3, 1991, Seattle, WA.

Landrum, P. F. and C. R. Stubblefield. 1991. Role of respiration in the accumulation of organic xenobiotics by the amphipod *Diporeia* spp. Environ. Toxicol. Chem. 10:1019-1028.

Landrum, P. F., B. J. Eadie, W. R. Faust, N. R. Morehead, and M. J. McCormick. 1983. Role of sediment in the bioaccumulation of benzo[*a*]pyrene by the amphipod, *Pontoporeia hoyi*. In: *Polynuclear Aromatic Hydrocarbons: Eighth International Symposium on Mechanism, Methods, and Metabolism*. M. W. Cooke and A. J. Dennis, eds. Batelle Press, Columbus, OH. pp. 799-812.

Landrum, P. F., S. R. Nihart, B. J. Eadie, and W. S. Gardner. 1984. Reverse-phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. Environ. Sci. Technol. 3:187-192.

Landrum, P. F., M. D. Reinhold, S. R. Nihart, and B. J. Eadie. 1985. Predicting the bioavailability of organic xenobiotics to *Pontoporeia hoyi* in the presence of humic and fulvic materials and natural dissolved organic matter. Environ. Toxicol. Chem. 4:459-467.

Landrum, P. F., S. R. Nihart, B. J. Eadie, and L. R. Herche. 1987. Reduction in bioavailability of organic contaminants to the amphipod *Pontoporeia hoyi* by dissolved organic material of sediment interstitial waters. Environ. Toxicol. Chem. 6:11-20.

Landrum, P. F., W. R. Faust, and B. J. Eadie. 1989. Bioavailability and toxicity of a mixture of sediment-associated chlorinated hydrocarbons to the amphipod *Pontoporeia hoyi*. In: *Aquatic Toxicology and Hazard Assessment: 12th Volume*. ASTM STP 1027. U. M. Cowgill and L. R. Williams, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 315-329.

Landrum, P. F., B. J. Eadie, and W. R. Faust. 1991. Toxicokinetics and toxicity of a mixture of sediment-associated polycyclic aromatic hydrocarbons to the amphipod *Diporeia* spp. Environ. Toxicol. Chem. 10:35-46.

Landrum, P. F., H. Lee II, and M. J. Lydy. 1992*a*.. Toxicokinetics in aquatic systems: Model Comparisons and use in hazard assessment. Environ. Toxicol. Chem. 11:1709-1725.

Landrum, P. F., T. D. Fontaine, W. R. Faust, B. J. Eadie, and G. A. Lang. 1992*b*. Modeling the accumulation of polycyclic aromatic hydrocarbons by the amphipod *Diporeia* (spp.). In: *Chemical Dynamics in Fresh Water Ecosystems*. F. A. P. C. Gobas and J. A. McCorquodale, eds. Lewis Publishers, Boca Raton, FL. pp. 111-128.

Landrum, P. F., B. J. Eadie, and W. R. Faust. 1992*c*. Variation in the bioavailability of polycyclic aromatic hydrocarbons to the amphipod *Diporeia* (spp.) with sediment aging. Environ. Toxicol. Chem. 11:1197-1208.

Laskowski-Hoke, R. A. and B. L. Prater. 1981. Dredged material evaluations: Correlations between chemical and biological evaluation procedures. Journal WPCF 53:1260-1262.

Laxen, D. P. H. 1985. Trace metal adsorption/coprecipitation on hydrous ferric oxide under realistic conditions: role of humic substances. Water Res. 19:1229-1236.

Lee, G. F. and R. A. Jones. 1984. Water quality significance of contaminants associated with sediments: An overview. In: *Fate and Effects of Sediment-Bound Chemicals in Aquatic Systems*. Proceedings of the Sixth Pellston Workshop, Florissant, CO, August 12-17, 1984. K. L. Dickson, A. W. Maki, and W. A. Brungs, eds. Pergamon Press, Elmsford, NY. pp. 3-34.

Lee, G. F., R. A. Jones, F. Y. Saleh, G. M. Mariani, D. H. Homer, J. S. Butler, and P. Bandyopadhyay. 1978. Evaluation of the elutriate test as a method of predicting contamination release during open water disposal of dredged sediment and environmental impact of open water dredged materials disposal, Vol. II - Data Report. Technical Report D-78-45. U. S. Army Engineer, Waterways Experiment Station, Vicksburg, MS.

Lee, H. II. 1991. A clam's eye view of the bioavailability of sediment-associated pollutants. In: *Organic Substances and Sediments in Water, Volume 3, Biological.* R. A. Baker, ed. Lewis Publishers, Chelsea, MI. pp. 73-93.

Lee H. II. 1992. Models, muddles, and mud: Predicting bioaccumulation of sediment-associated pollutants. In: *Sediment Toxicity Assessment.* G. A. Burton, Jr., ed. Lewis Publishers, Boca Raton, FL. pp. 267-293.

Lee H. II, B. L. Boese, and R. C. Randall. 1990. A method for determining gut uptake efficiencies of hydrophobic pollutants in a deposit-feeding clam. Environ. Toxicol. Chem. 9:215-219.

Leversee, G. J., J. P. Giesy, P. F. Landrum, S. Gerould, J. W. Bowling, T. E. Fannin, J. D. Haddock, and S. M. Bartell. 1982. Kinetics and biotransformation of benzo(*a*)pyrene in *Chironomus riparius*. Arch. Environ. Contam. Toxicol. 11:25-31.

Long, E. R., M. F. Buchman, S. M. Bay, R. J. Breteler, R. S. Carr, P. M. Chapman, J. E. Hose, A. L. Lissner, J. Scott, and D. A. Wolfe. 1990. Comparative evaluation of five toxicity tests with sediments from San Francisco Bay and Tomales Bay, California. Environ. Toxicol. Chem. 9:1193-1214.

Lopez, G. and R. Elmgren. 1989. Feeding depths and organic absorption for the deposit-feeding benthic amphipods *Pontoporeia affinis* and *Pontoporeia femorata*. Limnol. Oceanogr. 34:982-991.

Lydy, M. J., K. A. Bruner, D. M. Fry, and S. W. Fisher. 1990. Effects of sediment and the route of exposure on the toxicity and accumulation of neutral lipophilic and moderately water-soluble metabolizable compounds in the midge, *Chironomus riparius*. In: *Aquatic Toxicology and Risk Assessment: Thirteenth Volume.* ASTM STP 1096. W. G. Landis and W. H. van der Schalie, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 140-164.

Lydy, M. J., J. T. Oris, P. C. Baumann, and S. W. Fisher. 1992. Effects of sediment organic carbon content on the elimination rates of neutral lipophilic compounds in the midge (*Chironomus riparius*). Environ. Toxicol. Chem. 11:347-356.

Lydy, M. J. and P. F. Landrum. 1993. Assimilation efficiency for sediment sorbed benzo(*a*)pyrene by *Diporeia* spp. Aquat. Toxicol. 26:209-224.

Lynch, T. R. and H. E. Johnson. 1982. Availability of a hexachlorobiphenyl isomer to benthic amphipods from experimentally contaminated natural sediments. In: Aquatic Toxicology and Hazard Assessment: Fifth Conference. ASTM STP 766. J. G. Pearson, R. B. Foster, and W. E. Bishop, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 273-287.

Maaret, K., K. Leif, and H. Bjarne. 1992. Studies on the partition behavior of three organic hydrophobic pollutants in natural humic water. Chemosphere 24:919-925.

Mac, M. J., G. E. Noguchi, R. J. Hesselberg, C. C. Edsall, J. A. Shoesmith and J. D. Bowker. 1990. A bioaccumulation bioasay for freshwater sediments. Environ. Toxicol. Chem. 9:1405-1414.

Mackay, D. 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. 16:274-278.

Mackay, D. and S. Paterson. 1981. Calculating fugacity. Environ. Sci. Technol. 15:1006-1014.

Mackay, D. and S. Paterson. 1982. Fugacity revisited. Environ. Sci. Technol. 16:654A-660A.

Mackay, D. and S. Paterson. 1991. Evaluating the multimedia fate of organic chemicals: A level III fugacity model. Environ. Sci. Technol. 25:427-436.

Mackay, D., A. Bobra, and W. Y. Shin. 1980. Relationships between aqueous solubility and octanol-water partition coefficients. Chemosphere 9:701-711.

Malueg, K. W., G. S. Schuytema, D. F. Krawczyk, and J. H. Gakstatter. 1984. Laboratory sediment toxicity tests, sediment chemistry and distribution of benthic macroinvertebrates in sediments from the Keweenaw Waterway, Michigan. Environ. Toxicol. Chem. 3:233-242.

Malueg, K. W., G. S. Schuytema, and D. F. Krawczyk. 1986. Effects of sample storage on a copper-spiked freshwater sediment. Environ. Toxicol. Chem. 5:245-253.

Mancini, J. L. 1983. A method for calculating the effects on aquatic organisms of time varying concentrations. Water Res. 17:1335-1362.

Mayer, L. M. 1976. Chemical water sampling in lakes and sediments with dialysis bags. Limnol. Oceanogr. 21:909-911.

McCahon, C. P. and D. Pascoe. 1988. Culture techniques for three freshwater macroinvertebrate species and their use in toxicity tests. Chemosphere 17:2471-2480.

McCarthy, J. F. 1983. Role of particulate organic matter in decreasing accumulation of polynucular aromatic hydrocarbons by *Daphnia magna*. Arch. Environ. Contam. Toxicol. 12:559-568.

McCarthy, J. F. and B. D. Jimenez. 1985*a*. Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. Environ. Toxicol. Chem. 4:511-521.

McCarthy, J. F. and B. D. Jimenez. 1985*b*. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: Binding and dissociation. Environ. Sci. Technol. 19:1072-1076.

McCarthy, J. F. and M. C. Black. 1988. Partitioning between dissolved organic macromolecules and suspended particulates: Effects on bioavailability and transport of hydrophobic organic chemicals in aquatic systems. In: *Aquatic Toxicology and Hazard Assessment: 10th Volume*, ASTM STP 971. W. J. Adams, G. A. Chapman, and W. G. Landis, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 233-246.

McCarthy, J. F., B. D. Jimenez, and T. Barbee. 1985. Effect of dissolved humic material on accumulation of polycyclic aromatic hydrocarbons: Structure-activity relationships. Aquat. Toxicol. 7:15-24.

McCarty, L. S. 1990. A Kinetics-Based Analysis of Quantative Structure-Activity *Relationships in Aquatic Toxicity and Bioconcentration Bioassays with Organic Chemicals*. Ph.D thesis, Dept. of Biology, Univ. Waterloo, Ontario, Canada.

McCauley, D. J., P. Lea, P. Hoch, and B. Vitton. 1992. Comparison of whole sediment and sediment porewater toxicity to *Chironomus tentans* and *Hyalella azteca*. Abstracts, 13th Annual Meeting, Society of Environmental Toxicology and Chemistry, Cincinnati, OH. November 8-12, 1992.

McElroy, A. E. and J. C. Means. 1988. Factors affecting the bioavailability of hexachlorobiphenyls to benthic organisms. In: *Aquatic Toxicology and Hazard Assessment: 10th Volume*. ASTM STP 971, W. J. Adams, G. A. Chapman, and W. G. Landis, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 149-158.

McKim, J., P. Schmieder, and G. Veith. 1985. Absorption dynamics of organic-chemical transport across trout gills as related to octanol water partition-coefficient. Toxicol. Appl. Pharmacol. 77:1-10.

McMurthy, M. J., D. J. Rapport, and K. E. Chua. 1983. Substrate selection by tubificid oligochaetes. Can. J. Aquatic Sci. 40:1639-1646.

Microsoft Corp. 1991. *Microsoft® Excel User's Guide, Version 3.0*. Microsoft Corp., Redmond, WA.

Miller, G. C, V. R. Hebert, and W. W. Miller. 1989. Effect of sunlight on organic contaminants at the atmosphere-soil interface. In: *Reactions and Movement of Organic Chemicals in Soils*, B. L. Sawhney and K. Brown, eds. Soil Science Society of America, Inc. Madison, WI. pp. 99-110.

Moore, J. 1976. Importance of algae in the diet of subarctic populations of *Gammarus lacustris* and *Pontoporeia affinis*. Can. J. Zool. 55:637-641.

Muir, D. C. G., B. E. Townsend, and W. L. Lockhart. 1983. Bioavailability of six organic chemicals to *Chironomus tentans* larvae in sediment and water. Environ. Toxicol. Chem. 2:269-281.

Muir, D. C. G., G. P. Rawn, B. E. Townsend, W. L. Lockhart, and R. Greenhalgh. 1985. Bioconcentration of cypermethrin, deltamethrin, fenvalerate, and permethrin by *Chironomus tentans* larvae in sediment and water. Environ. Toxicol. Chem. 4:51-61.

Munawar, M., A. Murdroch, I. F. Munawar, and R. L. Thomas. 1983. The impact of sediment-associated contaminants from the Niagra River mouth on various size assemblages of phytoplankton. J. Great Lakes Res. 9:303-313.

Nebeker, A. V., M. A. Cairns, J. H. Gakstatter, K. W. Malueg, G. S. Schuytema, and D. F. Krawczyk. 1984. Biological methods for determining toxicity of contaminated freshwater sediments to invertebrates. Environ. Toxicol. Chem. 3:617-630.

Nebeker, A. V., S. T. Onjukka, M. A. Cairns, and D. F. Krawczyk. 1986. Survival of *Daphnia magna* and *Hyalella azteca* in cadmium-spiked water and sediment. Environ. Toxicol. Chem. 5:933-938.

Nebeker, A. V., G. S. Schuytema, W. L. Griffis, J. A. Barbitta, and L. A. Carey. 1989. Effect of sediment organic carbon on survival of *Hyalella azteca* exposed to DDT and endrin. Environ. Toxicol. Chem. 8:705-718.

Neely, W. B. 1979. Estimating rate constants for the uptake and clearance of chemicals by fish. Environ. Sci. Tech. 13:1506-1510.

Neely, W. B., D. R. Branson, and G. E. Blau. 1974. Partition coefficient to measure bioconcentration potential of organic chemicals in fish. Environ. Sci. Tech. 13:113-115.

Niimi, A. J. 1987. Biological half-lives of chemicals in fishes. Rev. Environ. Contam. Toxicol. 99:1-46.

Oliver, B. G. 1984. Uptake of chlorinated hydrocarbons from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. 41:878-883.

Oliver, B. G. 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol. 21:785-790.

Oliver, D. R. 1971. Life history of the Chironomidae. Ann. Rev. Entomol. 16:211-230.

Pascoe, D., A. F. Brown, B. M. J. Evans, and C. McKavanagh. 1990. Effects and fate of cadmium during toxicity tests with *Chironomus riparius* - The influence of food and artificial sediment. Arch. Environ. Contamin. Tox. 19:872-877.

Pavlou, S. P. and D. P. Weston. 1983. Initial evaluation of alternatives for developing sediment related criteria for toxic contaminants in marine waters. Puget Sound. Phase I. Development of Conceptual Framework. JRB Associates, Bellevue, WA.

Phelps, H. L. 1990. Development of an estuarine sediment burrowing bioassay for shipboard use. Bull. Environ. Contam. Toxicol. 45:722-728.

Phelps, H. L. and K. A. Warner. 1990. Estuarine sediment bioassay with oyster pediveliger larvae (*Crassostrea gigas*). Bull Environ. Contam. Toxicol. 44:197-204.

Phipps, G. L., G. T. Ankley, D. A. Benoit, and V. R. Mattson. 1993. Use of the aquatic oligochaete *Lumbriculus variegatus* for assessing the toxicity and bioaccumulation of sediment-associated contaminants. Environ. Toxicol. Chem. 12:269-279.

Pittinger, C. A., D. M. Woltering, and J. A. Masters. 1989. Bioavailability of sediment-sorbed and aqueous surfactants to *Chironomus riparius* (midge). Environ. Toxicol. Chem. 8:1023-1033.

Prater, B. L. and M. A. Anderson. 1977. A 96-hour sediment bioassay of Duluth and Superior harbor basins using *Hexagenia limbata, Asellus communis, Daphnia magna* and *Pimephales promelas* as test organisms. Bull. Environ. Contam. Toxicol. 13:159-169.

Presley, B. J., R. R. Brooks, and H. M. Kappel. 1967. A simple squeezer for the removal of interstitial water from ocean sediments. Journal of Marine Research 25:355-357.

Preull, R., J. L. Lake, W. Davis, and J. Quinn. 1983. Availability of polychlorinated biphenyls and polycyclic aromatic hydrocarbons in the blue mussel, *Mytilus edulis* from artifically resuspended esturaine sediment. Fourth International Ocean Disposal Symposium, Plymouth, England.

Quigley, M. A. 1988. Gut fullness of the deposit-feeding amphipod, *Pontoporeia hoyi* , in southeastern Lake Michigan. J. Great Lakes Res. 14:178-187.

Quigley, M. A. and H. A. Vanderploeg. 1991. Ingestion of live filamentous diatoms by the Great Lakes amphipod, *Diporeia* spp.: A case study of the limited value of gut contents analysis. Hydrobiologia 223:141-148.

Quigley, M. A., J. F. Cavaletto, and W. S. Gardner. 1989. Lipid composition related to size and maturity of the amphipod *P. hoyi*. J. Great Lakes Res. 15:601-610.

Rand, G. M. and S. R. Petrocelli. 1985. *Fundamentals of Aquatic Toxicology.* Hemisphere Publishing Corp., New York, NY.

Rasmussen, J. B. 1984. Comparison of gut contents and assimilation efficiency of fourth instar larvae of two coexisting chironomids, *Chironomus riparius* (Meigen) and *Glyptotendipes paripes* (Edwards). Can. J. Zool. 62:1022-1026.

Reichert, W. L., B. L. Eberhart, and U. Varanasi. 1985. Exposure of two species of deposit-feeding amphipods to sediment-associated [^{3}H] benzo[*a*]pyrene: Uptake, metabolism, and covalent binding to tissue macromolecules. Aquatic Tox. 6:45-56.

Robbins, J. A. 1986. A model for particle-selective transport of tracers in sediments with conveyor belt deposit feeders. Journ. Geophysical Res. 91:8542-8558.

Robbins, J. A. and J. Gustinis. 1976. A squeezer for efficient extraction of pore water from small volumes of anoxic sediment. Limnol. Oceanogr. 21:905-909.

Roemelt, P. M. and W. R. Seitz. 1982. Fluorescence polarization studies of perylene fulvic-acid binding. Environ. Sci. Technol. 16:613-616.

Royce, C. F. 1970. *An Introduction to Sediment Analysis.* Arizona State University, Tempe, AZ.

Rubenstein, N. I., C. N. D'Asaro, C. Sommers, and F. G. Wilkes. 1980. The effects of contaminated sediments on representative esturaine species and developing benthic communities. In: *Contaminants and Sediments. Volume 1.* R. A. Baker, ed. Ann Arbor Science Publishers, Ann Arbor, MI pp. 445-461.

Rubenstein, N. I., J. L. Lake, R. J. Pruell, H. Lee II, B. Taplin, J. Heltshe, R. Bowen, and S. Pavignano. 1987. Predicting bioaccumulation of sediment-associated organic contaminants: Development of a regulatory tool for dredged material evaluation. Internal report. U. S. EPA 600/x-87/368, Narragansett, RI. 54pp. + appendices.

Samoiloff, M. R., J. Bell, D. A. Birkholz, G. R. B. Webster, E. G. Arnott, R. Pulak, and A. Madrid. 1983. Combined bioassay-chemical fractionation scheme for the determination and ranking of toxic chemicals in sediments. Environ. Sci. Technol. 17:329-334.

SAS Institute. 1985. *SAS® User's Guide: Statistics, Version 5 Edition.* SAS Institute, Cary, NC.

SAS Institute. 1988. *SAS® User's Guide: Statistics, Version 5.18.* SAS Institute, Cary, NC.

Sasson-Brickson, G. and G. A. Burton, Jr.,1991. *In situ* and laboratory sediment toxicity testing with *Ceriodaphnia dubia.* Environ. Toxicol. Chem. 10:201-207.

Schlautman, M. A. and J. J. Morgan. 1993. Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials. Environ. Sci. Technol. 27:961-969.

Schlekat, C. E., B. L. McGee, and E. Reinharz. 1992. Testing sediment toxicity in Chesapeake Bay with the amphipod *Leptocheirus plumulosus*: An evaluation. Environ. Toxicol. Chem. 11:225-236.

Schrap, S. M. and N. Opperhuizen. 1990. Relationship between bioavailability and hydrophobicity: Reduction of the uptake of organic chemicals by fish due to the sorption on particles. Environ. Toxicol. Chem. 9:715-724.

Schubauer-Berigan, M. K. and G. T. Ankley. 1991. The contribution of ammonia, metals, and nonpolar organic compounds to the toxicity of sediment interstitial water from an Illinois River tributary. Environ. Toxicol. Chem. 10:925-939.

Schults, D. W., S. P. Ferraro, L. M. Smith, F. A. Roberts, and C. K. Poindexter. 1992. A comparison of methods for collecting interstitial water for trace organic compounds and metals analyses. Wat. Res. 26:989-995.

Servos, M. R. and D. C. G. Muir. 1989. Effect of dissolved organic matter from Canadian shield lakes on the bioavailability of 1,2,6,8-tetrachlorodibenzo-*p*-dioxin to the amphipod *Crangonyx laurentianus*. Environ. Toxicol. Chem. 8:141-150.

Servos, M. R. , D. C. G. Muir, and G. R. B. Webster. 1989. The effect of dissolved organic matter on the bioavailability of polychlorinated dibenzo-*p*-dioxins. Aquatic Tox. 14:169-184.

Siebert, P. C. 1977. Simple sedimentation methods, including the Andreason pipette and the Cahn sedimentation balance. In: *Particle Size Analysis.* J. D. Stockham and E. G. Fochtman, eds. Ann Arbor Science Publishers, Ann Arbor, MI, pp. 45-55.

Smith, J. H., W. R. Mabey, N. Bohonos, B. R. Holt, S. S. Lee, T. W. Chou, D. C. Bomberger, and T. Mill. 1977. Environmental pathways of selected chemicals in freshwater systems. Part I. Background and experimental procedures. EPA-600/7-77-113. U. S. Environmental Protection Agency, Washington, DC.

Spacie, A. and J. Hamelink. 1982. Alternative models for describing the bioconcentration of organics in fish. Environ. Toxicol. Chem. 1:309-320.

Stemmer, B. L., G. A. Burton, Jr., and S. Leibfritz-Frederick. 1990. Effect of sediment test variables on selenium toxicity to *Daphnia magna*. Environ. Toxicol. Chem. 9:381-389.

Swartz, R. C., D. W. Schultz, T. H. Dewitt, G. R. Ditsworth, and J. O. Lamberson. 1990. Toxicity for fluoranthene in sediment to marine amphipds: A test of the equilibrium partitioning approach to sediment quality criteria. Environ. Toxicol. Chem. 9:1071-1080.

Swindoll, C. M. and F. M. Applehans. Factors influencing the accumulation of sediment-sorbed hexachlorobiphenyl by midge larvae. Bull. Environ. Contam. Toxicol. 39:1055-1062.

Taghon, G. L., R. F. L. Self, and P. A. Jumars. 1978. Predicting particle selection by deposit feeders: A model and its implications. Limnol. Oceanogr. 23:752-759.

U. S. COE. 1976. *Ecological evaluation of proposed discharge of dredged or fill material into navigable waters.* Report #D-76-17. Environmental Effects Laboratory, U. S. Army Engineer Waterways Experiment Station, Vicksburg, MS.

U. S. EPA. 1978. *Bioassay Procedures for the Disposal Permit Program.* Ocean Disposal Working Group. Office of Research and Development. U. S. EPA-600/9-76/010. U. S. EPA, Gulf Breeze, FL.

U. S. EPA. 1989*a* . *Briefing Report to the EPA Science Advisory Board on the Equilibrium Partitioning Approach to Generating Sediment Quality Criteria.* U. S. EPA Office of Water Regulations and Standards. EPA 440/5-89-002. U. S. Environmental Protection Agency, Washington, DC.

U. S. EPA. 1989*b*. *EPA Guidance Manual: Bedded Sediment Bioaccumulation Tests.* EPA/600/x-89/302. U. S. Environmental Protection Agency, ERL-N, Pacific Ecosystems Branch, Newport, OR.

U. S. EPA. 1992. *Tiered testing issues for freshwater and marine sediments.* Workshop proceedings, Sept. 16-18, 1992. U. S. Environmental Protection Agency, Office of Water, Office of Science and Technology, and Office of Research and Development, Washington, DC.

U. S. EPA/COE. 1977. *Ecological evaluation of proposed discharge of dredged material into ocean waters.* Environmental Effects Laboratory, U. S. Army Engineer Waterways Experiment Station. U. S. Environmental Protection Agency, Vicksburg, MS.

U. S. EPA / U. S. COE. 1990. *Draft Ecological Evaluation of Proposed Discharge of Dredged Material into Ocean Waters.* EPA-503-8-90/002. U. S. Office of Marine and Estuarine Protection, Washington, DC.

Varanasi, U. 1989. *Metabolism of Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* CRC Press, Boca Raton, FL.

Veith, G. D., D. L. Defoe, and B. V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36:1040-1048.

Voice, T. C., C. P. Rice, and W. J. Weber, II. 1983. Effect of solids concentration on the sorptive partitioning of hydrophobic pollutants in aquatic systems. Environ. Sci. Technol. 17:513-518.

Walshe, B. M. 1951. The feeding habits of certain chironomid larvae (subfamily Tendipedinae). Proc. Zool. Soc. London 121:63-79.

Welch, H. 1968. Relationships between assimilation efficiencies and growth efficiencies for aquatic consumers. Ecology 49:755-759.

Wentsel, R., A. McIntosh, W. P. McCafferty, G. Atchison and V. Anderson. 1977*a*. Avoidance response of midge larvae (*Chironomus tentans*) to sediments containing heavy metals. Hydrobiologia 55:171-175.

Wentsel, R., A. McIntosh, and G. Atchison. 1977*b*. Sublethal effects of heavy metal contaminated sediment on midge larvae (*Chironomus tentans*). Hydrobiologia 56:153-156.

Wentsel, R., A. McIntosh, and W. P. McCafferty. 1978. Emergence of the midge *Chironomus tentans* when exposed to heavy metal contaminated sediment. Hydrobiologica 57:195-196.

Wiederholm, T., A. Wiederholm, and G. Milbrink. 1987. Bulk sediment bioassays with five species of fresh-water oligochaetes. Water, Air, and Soil. Pollut. 36:131-154.

Winsor, M., B. L. Boese, H. Lee II, R. C. Randall, and D. T. Specht. 1990. Determination of the ventilation rate of interstitial and overlying water by the clam *Macoma nasuta*. Environ. Toxicol. Chem. 9:209-213.

Witkowski, P. J., P. R. Jaffe, and R. A. Ferrara. 1988. Sorption and desorption dynamics of Aroclor 1242 to natural sediment. J. Contaminant Hydrol. 2:249-269.

Wolfe, N. L., R. G. Zepp, G. L. Baughman, R. C. Fincher, and J. A. Gordon. 1976. *Chemical and photochemical transformation of selected pesticides in aquatic systems.* EPA-600/3-76-067. U. S. Environmental Protection Agency, Washington, DC.

Wolfe, N. L., R. G. Zepp, and R. C. Hollis. 1978. Carbaryl, propham, and chloropropham: A comparison of the rates of hydrolysis and photolysis with the rate of biolysis. Water Res. 12:565-571.

Wood, L. W., G. Rhee, B. Bush, and E. Barnard. 1987. Sediment desorption of PCB congeners and their bio-uptake by dipteran larvae. Wat. Res. 21:875-884.

Wülker, W. and P. Götz. 1968. Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungzustandes von *Chironomus*-Larven (Diptera). Zeitschrift für Morphologie und Ökologie der Tiere 62:363-388.

Ziegenfuss, P. S.,W. J. Renaudette, and W. J. Adams. 1986. Methodology for assessing the acute toxicity of chemicals sorbed to sediments: Testing the equilibrium partitioning theory. In: *Aquatic Toxicology and Environmental Fate: Ninth Volume.* ASTM STP 921, T. M. Poston and R. Purdy, eds. American Society for Testing and Materials, Philadelphia, PA. pp. 479-493.

TABLE OF CONTENTS

Table of Contents (Continued)

Table of Contents (Continued)

LIST OF TABLES

List of Tables (Continued)

LIST OF FIGURES

PREFACE

The text is organized in the following manner: The first section provides an overall introduction to the work, including a literature review and the objectives of the research. The next four sections describe individual experiments conducted to meet the research objectives. The final section summarizes the conclusions of the previous sections and suggests future research needs and objectives pertainig to assessing the bioavailability of sediment-associated contaminants.

The four sections that describe individual experiments are written in styles to conform to publication guidelines for the *Journal of Environmental Toxicology and Chemistry* and *Chemosphere*. In this respect, they are self-contained papers with their own methods, results, and discussion sections.

A general "Literature Cited" section follows the appendix. This contains a listing of the references cited for all sections in the text.

INTRODUCTION

The Bioassay Approach to Sediment Toxicity

The field of sediment toxicology has evolved as mainly a descriptive science, and the method most commonly used in this discipline to describe and quantify sediment toxicity is the biological assay, or bioassay. Finney (1978) defines a bioassay as "an experiment for estimating the nature, constitution, or potency of a material (or of a process) by means of the reaction that follows its application to living matter." In this context, a bioassay involves a stimulus of a measured dose of the particular contaminant(s) in question which is applied to a biological organism. This dose, in turn, will elicit a response that is measured as the change in some biological characteristic or state (McCarty 1990).

Bioassays use two basic approaches, toxicity or bioaccumulation, to determine dose-response relationships. The toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus. From this, a specific endpoint is measured. For most toxicity tests, quantal responses (*e.g.*, all or none, alive or dead) are measured and reported as effective or lethal concentrations (*i.e.*, LC_{50} or LOEL values), although other endpoints such as inhibition of growth and development and alterations in behavior can be measured. Bioaccumulation assays focus not on specific endpoints but on the kinetics of the toxicologic process itself, to include the absorption, distribution, biotransformation, and elimination of the chemical in question. Data from bioaccumulation assays can thus be used to develop toxicokinetic models for estimating and predicting contaminant accumulation in organisms.

The field of sediment toxicology is rather new. It evolved in the United States from the need to control elevated levels of contaminants in dredged sediments in the late 1960s. At that time, the U. S. Army Corps of Engineers (COE) was routinely dumping large quantities of dredge spoils into open waterways (Lee and Jones 1984). Because of the potential effect of these dredge spoils on water quality, the Federal Water Quality Administration (a predecessor of the U. S. Environmental Protection Agency, U. S. EPA) developed a set of criteria (Jensen criteria, Boyd *et al.* 1972) that required certain parameters such as copper, COD, zinc, and volatile solids to be monitored in dredged sediment. If the concentration of these parameters exceeded the criterion value, then the sediment was deemed unfit for open water disposal, and alternate disposal methods had to be found. Evaluations made under these criteria

showed that a large portion of the dredged sediment disposed of in open waterways was, indeed, unfit for disposal. However, the Jensen criteria assumed that the total concentration of contaminants in bulk sediment was equal or proportional to what could be released as the bioavailable fraction into the water column, an assumption that did not accurately estimate bioavailability of contaminants to the aquatic ecosystem. To remedy this situation, the federal Congress authorized the Dredged Material Research Program (DMRP) in the 1970s. This program studied a variety of environmental aspects of dredged sediment disposal (Lee and Jones 1984). One principal result was the development of the standard elutriate test for determining the bioavailable fraction of released contaminants from dredge spoils. This test was developed to simulate the type and duration of sediment/water contact and mixing that would be expected to occur during hydraulic dredging operations (Jones and Lee 1978, Lee *et al.* 1978).

From the results of this work and additional government-sponsored research, elutriate tests were found to generally predict the direction and approximate magnitude of release of contaminants from dredged materials. The elutriate test was therefore deemed a good tool for predicting the potential for release of contaminants from sediments that are stirred into the water column. This research also determined that, contrary to Jensen criteria assumptions, bulk properties of sediments cannot be used to estimate the release of contaminants from them. Subsequent to this research, the U. S. EPA and the COE published the Marine Protection, Research, and Sanctuaries Act (Section 103) Implementation Manual for evaluating ecological effects of dredged material disposal (U. S. EPA/ COE 1977). In addition to the use of whole sediment and suspended sediment exposures, this manual described methods used in sediment toxicity assessment that incorporated the use of elutriate bioassays.

In the late 1970s and early 1980s the scientific community was concerned that toxicity testing conducted with bulk sediment, "standard" elutriates and other aqueous extracts did not adequately describe the availability of many sediment-associated contaminants to aquatic organisms (Brannon *et al.* 1980, Engler 1980, Anderson *et al.* 1984). One shortcoming of the elutriate tests developed in the DMRP program was the inability to specify the redox conditions that must prevail during a bioassay. Since redox conditions determine the bioavailable fraction of many ionic species (*i.e.*, metals, ammonia), the bioavailability of these contaminants would be largely determined by the conditions at the dredging and disposal sites. Even though elutriate bioassays may have been conducted under "oxic" or "anoxic" conditions in

the laboratory, results of these bioassays could not be directly compared to field conditions. Alternatively, sediment porewaters were given attention as possible indicators of contaminant bioavailability for in-place pollutants as well as in dredging operations (Bahnick *et al.* 1981). In 1981, sediment quality indicators were proposed that compared interstitial water (porewater) contaminant concentrations with the U. S. EPA's water quality criteria via equilibrium partitioning theory (Burton 1991). Basically, this approach suggested that interstitial water concentrations of contaminants could be related to bulk sediment concentrations, and biological effects on sediment-dwelling organisms could be correlated to these interstitial water concentrations (U. S. EPA 1989*a*). Bioassays were later developed that used porewater extracted from contaminated sediments to test dose-response relationships with a number of indicator organisms.

Bioassays Performed with Bulk Sediment

Numerous toxicity studies conducted with bulk sediments have been reported in the literature (see Giesy and Hoke 1989 for a review). Mortality after 24-h to 10-d exposures are the most common endpoints for these studies (Adams *et al.* 1984, 1986; Cairns *et al.* 1984, Malueg *et al.* 1984, 1986, Nebeker *et al.* 1986, Ziegenfuss *et al.* 1986, Becker *et al.* 1990, Swartz *et al.* 1990, ASTM 1992*a,b*), although other endpoints such as sediment avoidance and burrowing speed (Wentsel *et al.* 1977*a*, Phelps 1990), inhibition of growth or emergence (Wentsel *et al.* 1977*b*,1978, Nebeker *et al.* 1984, Adams *et al.* 1986, Giesy *et al.* 1990), and reproduction (Schlekat *et al.* 1992) have been examined. Indicator organisms used in freshwater sediment toxicity bioassays have included species ranging from microorganisms (*Photobacterium phosphoreum*, with the Microtox ® bacterial assay, Brouwer *et al.* 1990) to aquatic vertebrates (fish species *Pimephales promelas, Salmo gairdneri, Micropterus salmoides, Carassius auratus*; Prater and Anderson 1977, Birge *et al.* 1984, Francis *et al.* 1984). For evaluating bulk sediment toxicity, the most commonly used organisms have been the macroinvertebrates. Members of the family Crustacea, such as the amphipods *Gammarus pulex, Hyallella azteca*, (freshwater), *Rehpoxynius abronius* (marine) and cladocerans *Daphnia magna, D. pulex*, and *Ceriodaphnia dubia*, (all freshwater species) have been used in numerous toxicity tests (Malueg *et al.* 1984, Nebeker *et al.* 1984, Long *et al.* 1990, Sasson-Brickson and Burton, 1991). Other commonly used freshwater indicator organisms have included midge larvae (*i.e., Chironomus tentans* and *C. riparius*), mayfly larvae (*Hexagenia* spp.), molluscs (*Corbicula leana, Crassostrea gigas*), and the oligochaete worms, *Lumbriculus variegatus, Stylodrilus*

heringinus (freshwater), and *Capitella capitata* (marine; Burres and Chandler 1976, Wentsel *et al.* 1977*a,b*, 1978, Bailey and Liu 1980, Cairns *et al.* 1984, Chapman and Fink 1984, Graney and Giesy 1988, Ingersoll and Nelson 1990, Keilty and Landrum 1990, Phelps and Warner 1990, Phipps *et al.* 1993). In addition, a multitude of bioaccumulation bioassays utilizing bulk sediment have been conducted with freshwater and marine species (Halter and Johnson 1977, Landrum *et al.* 1983, Muir *et al.* 1983, 1985, Oliver 1984, Reichert *et al.* 1985, Swindoll and Applehans 1987, Knezovich and Harrison 1988, Gobas *et al.* 1989*a*, Pittinger *et al.* 1989, Fry and Fisher 1990, Lee *et al.* 1990, Mac *et al.* 1990).

A number of laboratory techniques have been used with bulk sediment. Early studies in the 1970s employed static tests patterned after tried and true methods of ambient water toxicity testing (Wentsel 1977*a,b*). Later studies employed flow-through techniques with regular exchanges of fresh overlying water at regular intervals. Static and static renewal methods were also popular for both toxicity and bioaccumulation asssays that used either historically contaminated sediments or laboratory spiked sediments (U. S. EPA 1978, Rubenstein *et al* 1980, 1987, Landrum 1989, Ferraro *et al* 1990). In recent years, the American Society for Testing and Materials (ASTM) has approved two standard guides that provide general guidelines for conducting whole sediment toxicity tests with marine and freshwater macroinvertebrates as well as a guide for the proper collection, storage, and manipulation of sediments (ASTM 1992 *a,b,c*). These guides suggest the use of static test procedures for conducting toxicity tests with marine and estuarine amphipods, and either static or flow-through designs for testing the toxicity of freshwater sediments. Other recent bioaccumulation assay guidelines suggest the use of either flow-through or static designs (U. S. EPA 1989*b*).

Techniques that determine either bioaccumulation or toxicity in bulk sediments pose problems that are unique to bulk sediment exposures. Unlike aqueous extracts and porewater that can be readily diluted with ambient water, bioassays that utilize whole sediments must use sediment dilutions in order to determine dose-response relationships. It has been suggested that samples be diluted with a reference sediment that is not as contaminated as the sediment to be tested (Chapman 1988). What constitutes an acceptable reference or control sediment and the availability of such a sediment remains problematic. Given that such a reference sediment can be obtained, additional questions concerning the proper collection, storage, mixing, and length of equilibration time arise. Diluted sediments have undergone physical and chemical changes. Altered properties of the sediment such as organic carbon content

and particle-size distribution can affect bioaccumulation and toxicity. Thus, accurately estimating the availability of the contaminant(s) to the indicator species becomes impossible, and the validity of the results obtained from such bioassays is doubtful.

In the case of metals and other ionic contaminants, the proper collection and storage of sediments is critical (Burton 1991). The bioavailability of metals such as cadmium and selenium is greatly altered upon disturbance of redox gradients and volatilization of sulfides from the bulk sediment (DiToro *et al.* 1990, Stemmer *et al.* 1990). In this respect, the mixing process during sediment dilution alone may alter toxicity data. Therefore, aqueous extracts of contaminated sediments, such as elutriates or extracted porewater have been used as substitutes for bulk sediment bioassays.

The Use of Elutriates

The elutriate test, as developed by the U. S. Army Corps of Engineers, involved the mixing of one volume of wet sediment with four volumes of water for 30 minutes, settling for 1 hour, and filtration of the supernatant (U. S. COE 1976). The supernatant was then used as an aqueous medium for standard toxicity bioassays. Procedures involving the mixing, settling, and filtration of the sediment/water mixture were established to simulate settled sediment slurries after hydraulic dredging. Results obtained from numerous toxicity bioassays conducted by the Corps of Engineers are reviewed by Lee *et al.* 1978. In short, exposure to sediment elutriates from "highly" contaminated areas caused 20 to 50% of the indicator species to die within a four-day period (Lee and Jones 1984). As previously mentioned, these studies gave only a rough estimation of the bioavailability of contaminants released in dredging operations, since laboratory conditions generally did not mimic conditions found in the field. Neither were the contaminants that caused the toxicity identified. Consequently, these "kill 'em and count 'em" techniques did little to describe the exposure of sediment-associated contaminants.

Numerous variations of the original elutriate standard procedure have been implemented (Chapman and Fink 1984, Dutka and Kwan 1988, Athey *et al.* 1989, Daniels *et al.* 1989, Hoke *et al.* 1990). Variations on the method and length of time for mixing and settling, and whether to filter or centrifuge the resulting supernatant have been examined by various laboratories to answer specific questions about the bioavailability and route of accumulation of sediment-associated contaminants. Since filtration may significantly reduce the bioavailability of some hydrophobic

chemicals, centrifugation has been used to remove the particulate fraction in some studies (Chapman and Fink 1984, Sasson-Brickson and Burton 1991).

Elutriate tests have been expanded to assess not only resuspended sediments at dredging sites, but also to evaluate toxicity and bioaccumulation of non-suspended sediments (Munawar *et al.* 1983, Anderson *et al.* 1984, Giesy *et al.* 1988). In recent years, various extractable fractions of sediment/water dilutions have been implemented for use in toxicity identification evaluation (TIE) by the U. S. EPA (Ankley *et al.* 1991). These procedures selectively fractionate a prepared elutriate for the purpose of identifying its toxic componets. One study compared a standard elutriate with one treated to remove dissolved metals and found that uptake of ^{14}[C]-$NaHCO_3$ by phytoplankton was greater in the standard elutriate than in the treated elutriate (Munawar *et al.* 1983). The authors concluded that synergistic effects of nutrients, metals, and organic compounds determined the bioavailability of the contaminants contained in the elutriates.

A few studies have directly compared exposure to elutriates with porewater or bulk sediment as test treatments. In these toxicity assays, elutriates were found to be less toxic (Ankley *et al.* 1991, Sasson-Brickson and Burton 1991), more toxic (Hoke *et al.* 1990), or comparable in toxicity to other fractions (Laskowski-Hoke and Prater 1981, Chapman and Fink 1984). Other studies have been conducted as comparisons to detect sensitivity of the indicator species or test methods (*i.e.*,, Microtox® with elutriate vs. *C. riparius* 10-day survival test with bulk sediment; Nebeker *et al.* 1984, Giesy *et al.* 1988, Athey *et al.* 1989, Burton *et al.* 1990, Long *et al.* 1990). Studies of this nature, however, do not directly compare test phases, and thus the appropriateness of using a standard elutriate as a substitute for bulk sediment assays cannot be evaluated.

Bioassays Using Porewater

A popular bioassay approach for assessing the toxicity of contaminated sediments has been to expose organisms to extracted porewater (interstitial water). However, the concentration of contaminants in porewater depends upon the method by which it is collected and stored. Numerous methods on the extraction of porewater have been reported in the literature. Some of the most popular methods are those of squeezing (Presley *et al.* 1967, Robbins and Gustinis 1976, Bender *et al.* 1987, Carr *et al.* 1989), dialysis (Mayer 1976, Hoepner 1981, Carignan 1984), centrifugation with or without subsequent filtration of the supernatant (Edmunds and Bath 1976, Bahnick *et al.* 1981, Landrum *et al.* 1987, Giesy *et al.* 1988, 1990), and various *in situ* methods

(Barnes 1973, Hesslein 1976, Brinkman *et al.* 1982, Bottomley and Bayly 1984). Although a number of toxicity tests have been conducted that used porewater obtained by various methods, only a few studies have actually compared the concentration and/or availability of contaminants among extraction methods. One such study concluded that the method used in collecting porewater can profoundly affect the concentrations of constituents such as dissolved organic carbon and volatile species (Howes and Wakeham 1985). Another study concluded that parameters such as total organic carbon, particle-size distributions and ionic species can vary with the extraction technique used (Laxen 1985). However, a study that compared centrifugation followed by filtration to that of dialysis yielded similar concentrations of metals (Carignan *et al.* 1985).

Several studies have shown that the toxicity of contaminants in porewaters versus whole sediments is significantly different. An assay that compared porewater to whole sediment exposures with mayfly larvae found that organisms were less sensitive to whole sediment than porewater (Giesy *et al.* 1990). Tests conducted with *Cereodaphnia dubia* showed that survival in porewater was slightly lower than in whole sediment exposures (Sasson-Brickson and Burton 1991). However, LC_{50} values for midge larvae were similar in whole sediment contaminated with zinc acetate and porewater extracted from it (McCauley *et al.* 1992).

From data generated in porewater assays, the bioavailability of sediment-associated contaminants apparently depends upon the chemical and physical properties of both the sediment and the contaminants. In particular, the partitioning of metal species via changes in oxygen/redox gradients, pH, temperature, complexation, and precipitation is altered upon extraction of porewater (Forstner 1990, Burton 1991). Unless these factors are controlled and porewater extraction and storage methods are standardized, bioassays that use porewater as substitutes for metal contaminated whole sediment exposures may not reflect the same or similar exposure of the bulk sediment.

The partitioning of non-ionic organic contaminants between particles and water has been examined in numerous studies and is relatively better understood than that of ionic species. Presently, a standard model exists for describing the partitioning process of hydrophobic organics that incorporates the octanol-water partition coefficient. This model is the basis for equilibrium partitioning theory (U. S. EPA 1989*a*). The U. S. EPA currently supports the equilibrium partitioning approach to generating sediment quality criteria, based on the theory that neutral organic chemicals sorbed to sediments achieve thermodynamic equilibrium between

sediment and pore water over relatively short periods of time (U. S. EPA 1989*a*, DiToro *et al.* 1991). At equilibrium, the concentration of contaminant in either phase can be estimated by measuring the mass in the other phase, normalized to its concentration of organic carbon. This idea tends to be appropriate for neutral hydrophobic contaminants that bind tightly to organic carbon, causing the amount of organic carbon to be the controlling factor in partitioning of the contaminants. Work done by Pavlou and Weston (1983) showed that porewater concentrations could be estimated via equilibrium partitioning. Adams *et al.* (1984) later demonstrated that kepone toxicity in the midge could be correlated to the porewater concentration via the carbon-normalized sediment concentration. Their results suggested that porewater was the primary route of exposure to the organism. However, recent studies suggest exposure from additional routes such as sediment particles and colloids for some species (Cairns *et. al.* 1984, Landrum 1989, Landrum *et al.* 1989). Thus, the relationship between porewater and sediment may not be as simple as equilibrium partitioning theory suggests when a substantial amount of exposure comes from sources other than porewater.

A more accurate description of uptake routes needs to be made to adequately determine and compare dose-response relationships between whole sediment and aqueous extracts, To this end, the kinetic approach is a more direct approach to describe the various mechanisms of accumulation. Although the toxicity assays done in the past have provided a good starting point for these tasks, the use of bioaccumulation assays will increase our understanding of the basic processes involved in uptake of sediment-associated contaminants. From the literature previously reviewed, the utility and limitations of using porewater and elutriates as substitutes for whole sediment exposures have not been adequately researched. Where differences in accumulation and/or toxicity of sediment-associated contaminants occur among the three media, an investigation of exposure needs to be undertaken to determine the reasons for these differences. A thorough knowledge of bioaccumulation processes and the mechanism(s) for routes of exposure needs to be obtained before this question can be answered.

Toxicokinetics

Some Basic Concepts

Kinetic approaches are a way to study bioaccumulation or rate processes of contaminant uptake in an organism. These processes - accumulation, distribution, metabolism, and elimination - are used to construct mathematical models that

interpret the results of such bioassays. Rates of contaminant uptake can then be used to predict bioaccumulation of contaminants in biota that ultimately affect ecosystem health and diversity.

Toxicokinetic models deal with data obtained over the interval described in a typical uptake curve (Figure 1.1). Unlike steady-state models that only describe that portion of the uptake curve where the flux of contaminant into the organism is equal to that going out of the organism (the plateau in the uptake curve), kinetic models can predict non-steady state conditions and multiple uptake routes (Landrum *et al.* 1992*a*). Kinetic models were first used in aquatic toxicology to describe the uptake kinetics observed in fish. Simple first-order kinetic models have been used by a number of researchers to describe general uptake processes from water-only systems (Neely *et al.* 1974, Spacie and Hamelink 1982, Mancini 1983, Chew and Hamilton 1985, Connolly 1985, Niimi 1987, Landrum *et al.* 1992*b*). Bioassays used in these studies have generally followed the procedure of measuring accumulation of a contaminant in an organism over predetermined timed intervals. After the uptake phase, an elimination curve can be generated by placing the organism into an uncontaminated medium where contaminant concentration in the organism is again monitored over time. A pictoral representation of the first-order kinetic model is shown in Figure 1.2. The general form of the equation used for uptake is

$$\frac{dC_a}{dt} = k_u C_w - k_e C_a \qquad (1)$$

and for elimination

$$\frac{dC_a}{dt} = -k_e C_a \qquad (2)$$

where

k_u = the uptake rate coefficient ($ml \cdot g^{-1} \cdot h^{-1}$),

C_w = concentration of a compound in water ($g \cdot ml^{-1}$),

C_a = concentration of a compound in an organism ($g \cdot g^{-1}$),

k_e = the elimination rate coefficient (h^{-1}), and

t = time (h)

with the integrated form for uptake

$$C_a = C_w \frac{k_u}{k_e} * [1 - e^{(-k_e t)}] \qquad (3)$$

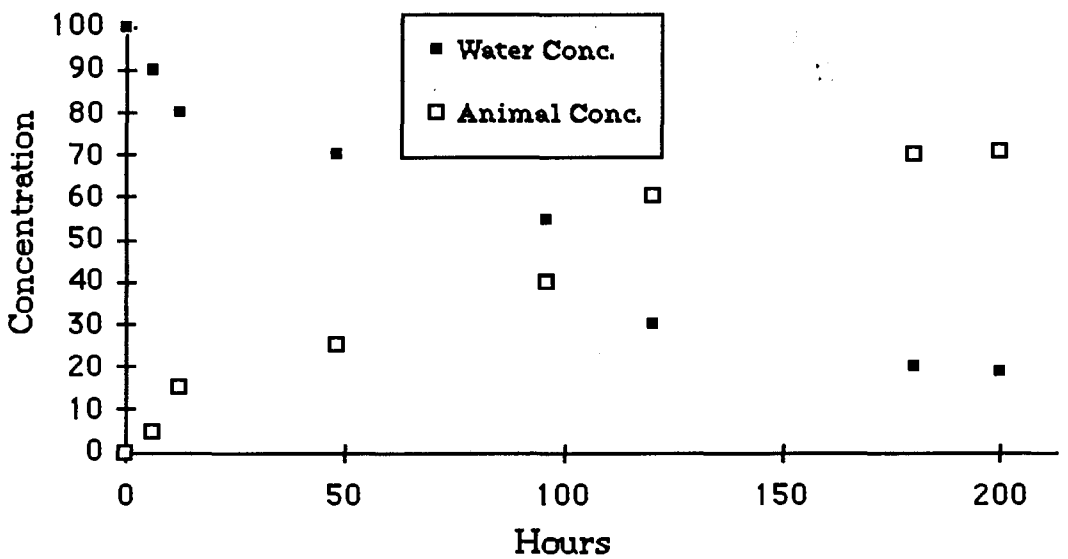

Figure 1.1. Typical first-order uptake and elimination curve.

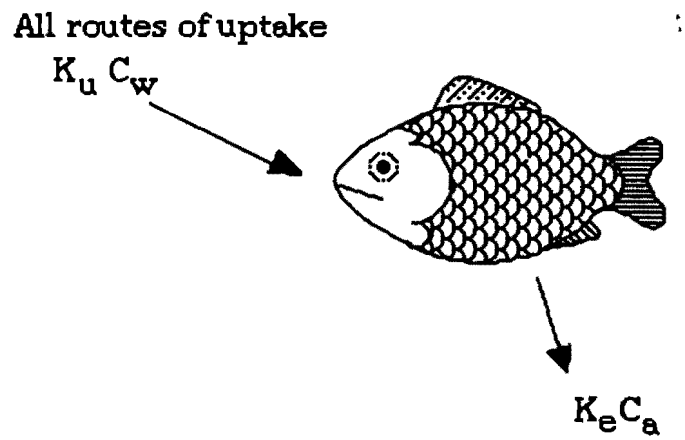

Figure 1.2. First-order kinetic model for bioaccumulation in a water-only exposure.

and for elimination

$$C_a = C_{a_o}(e^{-k_e t}) \quad (4)$$

where

C_{a_o} = the concentration of a compound in an organism at the beginning of the elimination period.

During the initial portion of the uptake curve when C_a is very small, the increase in tissue residues with time should be linear (Figure 1.1). In this portion of the curve, then, the term representing the elimination from the organism, $-k_d C_a$, can be deleted. This simplifies the integrated form of the equation to

$$C_a = k_u C_w t. \quad (5)$$

As C_a becomes larger, the flux out of the organism, $k_e C_a$ increases until it balances the flux into the organism, $k_u C_w$. This is shown in Figure 1.1 as a plateau in the uptake curve, which signifies that a steady-state has been reached in the organism. At steady-state, the rate of elimination is balanced by the rate of uptake, so that $k_u C_w = k_e C_a$. However, steady-state should not be considered a thermodynamic equilibrium condition. Steady-state conditions represent a constant concentration of a compound in an organism as a result of energy put in to the system (*i.e.*, the rate of compound going in to the organism is the same as that going out of the organism). This should be distinguished from equilibrium conditions, where the free energy of the system is equal to zero, and no energy is required to maintain the state (Landrum and Lydy 1991). At steady-state, the concentration of contaminant in the organism is compared to the concentration in the water as a ratio, or bioconcentration factor (BCF). The BCF is then equivalent to C_a / C_w, which equals k_u / k_e. In such systems where contaminants are acquired from additional sources (*i.e.*, sediment, porewater, added food), the term bioaccumulation factor (BAF) is used.

Toxicokinetic Models Currently Available

First-order kinetic models have been described in two ways according to the exposure system used (Landrum *et al.* 1992*a*). *Mass balance* models are used in closed or static bioassays where there is no gain or loss of contaminant to the system over the testing interval. This system can be described as having two compartments, a water compartment and an animal compartment. Only the disposition of the contaminant changes with time, so that a steady decrease in contaminant concentration from water simultaneously follows a steady increase in animal

concentration until steady state is obtained (Figure 1.1). Mathematically, the mass balance model is

$$\frac{dQ_a}{dt} = (k_{um}Q_w) - (k_eQ_a) \quad (6)$$

where

Q_a = the mass of compound in the organism (g)

k_{um} = the conditional mass based uptake rate constant (h^{-1}), and

Q_w = the mass of compound in the water (g).

Because Q_w is a function of time in this closed system, the differential equation has no exact solution. The way to get around this problem is to assume that the mass of compound in the system is conserved at some value A. The mass of A is only distributed between the water and animal compartment of the system and can be described as

$$A = Q_a + Q_w. \quad (7)$$

The original mass balance equation

$$\frac{dQ_a}{dt} = (k_{um}Q_w) - (k_eQ_a) \quad (6)$$

then becomes

$$\frac{dQ_a}{dt} = [k_{um}(A - Q_a)] - [k_eQ_a] \quad (8)$$

upon substitution of Q_w. Upon integration, this equation becomes

$$Q_a = \frac{[(k_{um}A)(1 - e^{-(k_{um} + k_e)t})]}{(k_{um} + k_et)}. \quad (9)$$

An assumption of the above model is that there is no biotransformation, metabolism, or other loss of compound to the system. As the elimination term, k_e is minimal, the above integrated form of the equation simplifies to

$$Q_a = A\,[1-e^{(-k_{um}t)}]. \quad (10)$$

The second type of first-order kinetic model is the *constant infusion* model, used for data generated in a flow-through system. In such a system, the concentration of contaminant in the water stays constant over time, and the assumptions of mass balance are not used. However, as with the mass-based model, the assumption can be made that C_a is approximately equal to zero in the first part of the uptake curve, so that a simplification of equation (1) can be made that, after integration, becomes

$$C_a = k_uC_wt. \quad (11)$$

First-order rate models have been the most popular ways to interpret data from sediment/water bioaccumulation assays. Uptake rate coefficients have been determined for a variety of sediment-associated contaminants in animals ranging from fish (Neely 1979), to midge larvae (Leversee *et al.* 1982, Lydy *et al.* 1992), to amphipods and mysids (Evans and Landrum 1989, Landrum 1989, Landrum *et al.* 1992*a*). Numerous other toxicokinetic models based on fugacity, physiological and energetics, and bioenergetics have been used in aquatic toxicology for both water-only and water/sediment systems (Barron *et al.* 1990, Lake *et al.* 1984, Landrum *et al.* 1992*c*, Lee 1992, Gobas and Mackay 1987, Gobas *et al.* 1988, 1989*a*, Ferraro *et al.* 1990, Mackay and Paterson 1981, 1982, 1991). Such models incorporate a variety of variables and assumptions that are dependent upon experimental design and available data.

The Bioavailability of Non-polar Organic Xenobiotics

Regardless of the kinetic model used, the basic assumptions of the model must be followed. As previously stated, an assumption of the mass balance kinetic model is that no biotransformation or metabolism of the contaminant occurs during an assay. If, however, this assumption is overlooked, the interpretation of data will be in error. For example, if the uptake of two contaminants is examined in separate bioaccumulation assays under the same exposure conditions (*i.e.*, contaminant concentrations are similar), but kinetic results are different, a difference in potency of the contaminants would be assumed. However, if one contaminant was metabolized and the other was not, the same dose of parent compound was likely not available to the target tissue in both cases, and exposure conditions were not the same in both assays. A higher dose of the metabolized contaminant would need to be present for the same effect to be seen in both assays.

For a contaminant to elicit a response, the contaminant must come into contact and react with an appropriate receptor site on or in the organism at a high enough concentration and for a sufficient length of time. Because bioaccumulation and toxicity bioassays are only surrogates for dose or concentration-response relationships (*i.e.*, the amount of toxicant at the actual site(s) of toxic action in the organism is not known), their interpretation is dependent upon the routes of exposure to the organism. Exposure is a function of the bioavailability of a compound. Landrum and Robbins (1990) define bioavailability as the fraction of total contaminant that is available for accumulation via all existing routes open to the organism. In this sense, exposure is not dependent upon the total concentration

of contaminant in the system, but only that fraction that can be adequately taken up and assimilated by the organism. The question then becomes, "Just what is biologically available to an organism placed into a bioassay situation?" Many factors associated with this problem have been presented and are addressed in various review articles (Adams 1987, Knezovich *et al.* 1987, Landrum and Robbins 1990, Lee 1991). These factors can be grouped into those associated with characteristics of the contaminant (chemical and physical properties), characteristics of the environment (sediment/water characteristics), and characteristics of the biota. The interactions of the factors involved in each of these groups, then, determine the amount of contaminant that is available for uptake and accumulation in any particular indicator species of concern.

Characteristics of the Contaminant

The bioavailability of a contaminant in aquatic systems is frequently dependent upon the amount that is freely dissolved in the aqueous environment (Landrum *et al.* 1985, McCarthy *et al.* 1985, Rand and Petrocelli 1985, Black and McCarthy 1988). For nonpolar organic xenobiotics, the freely dissolved portion may be extremely reduced by the binding to organic material, dissolved organic material (DOM), and particulate organic material (POC). The greatest factor in determining the bioavailability of such compounds is that of hydrophobicity, expressed as the octanol-water partition coefficient, K_{ow}. This binding of an organic compound to organic material is proportional to its log K_{ow} (Bysshe 1982, Mackay 1982, Gobas and Mackay 1987, Wood *et al.* 1987). A number of researchers have directly correlated bioaccumulation factors of fish (Neely *et al.* 1974, Chiou *et al.* 1977, Veith *et al.* 1979, Kenaga and Goring 1980, Gobas *et al.* 1989*b*), mussels (Ernst 1977, Geyer *et al.* 1982, Pruell *et al.* 1983), oligochaete worms (Oliver 1987), and amphipods (Landrum 1989) to log K_{ow} values up to approximately six. The rise in accumulation rates with increasing log K_{ow} are thought to be due to sorption characteristics of the compound. Above log K_{ow} values of six, accumulation has been found to decline in oligochaetes, apparently due to sorption properties and changes in uptake and elimination of the contaminant (Oliver 1984, 1987). Contaminants with a low log K_{ow} are less strongly sorbed to organic material, and are thus more available for uptake by an organism. However, the same compounds are more rapidly eliminated by the animal than are compounds of higher log K_{ow}, resulting in low accumulation factors (Landrum and Robbins 1990). Further, compounds with a log K_{ow} greater than 6 or 7 are less bioavailable, due to slower desorption from particles and reduced

transport across membranes (McKim *et al.* 1985, Landrum 1989). Thus, partitioning of organic contaminants into or out of an organism is apparently driven primarily by lipophilicity.

The bioavailability of hydrophobic organic contaminates depends not only on log K_{OW}, but may also be a function of molecular structure. For instance, the uptake coefficients for chlorinated compounds from sediments are much greater than for unchlorinated compounds of the same solubility (*i.e.*, K_{OW}; Landrum 1989, Landrum *et al.* 1989).

Transformation potential will greatly alter the bioavailability of a contaminant in the aquatic environment. Transformation can occur via oxidation, hydrolysis, or photolysis, as well as by metabolic processes in biota. Oxidation reactions occur through the interaction of substances with free radicals naturally occurring in water, and hydrolysis has been found to play a significant role in the environmental degradation of some pesticides (Wolfe *et al.* 1976, 1978). Photolysis is an important route for the degradation of some nonpolar organics. Photolysis rates are dependent upon the contaminant's position in the aquatic environment. For example, in the aqueous environment, benzo(*a*)pyrene (BaP) exhibited a half-life of less than 3 hours, based on calculated values (Smith *et al.* 1977). However, such compounds sorbed to sediment particles undergo little, if any, degradation due to the substantial light attenuating affect of the particles and possible photochemical quenching of electronically excited states on surfaces of the particles (Miller *et al.* 1989). Photolysis of compounds sorbed to sediment is also a function of the depth and turbidity of the water column above it.

Loss of contaminant from the system will also affect its bioavailability. High volatility will result in the loss of contaminant from either the sediment or aqueous phase, and will render the compound unavailable for uptake. Many hydrophobic contaminants possess vapor pressures sufficiently high to make their escape into the atmosphere a major pathway of loss to the aquatic environment (Klopffer *et al.* 1982).

Other chemical and physical characteristics that can alter bioavailablility include chelation, complexation, ionization, and cation exchange. Although these factors can be responsible for significantly limiting the availability of ionic and inorganic contaminants to biota, they play a limited role in altering bioavailability of hydrophobic organics.

Characteristics of the Environment

The predominant environmental factor affecting the bioavailability of hydrophobic organic contaminants is adsorption to particles and organic material. Sediment composition along with its particle size distribution plays a key role in the bioavailability of hydrophobic organics, as numerous studies have confirmed (see Adams 1987 and Landrum and Robbins 1990 for a review). A number of studies have shown that accumulation in animals is a direct function not only of the amount of particulate matter present, but of the amount and type of organic material present (McCarthy 1983, Muir *et al.* 1983, Hall *et al.* 1986, Capel and Eisenreich 1990, Lydy *et al.* 1990, Schrap and Opperhuizen 1990). In a sediment/water system, hydrophobic contaminants are bound tightly to particles, making them unavailable for accumulation by biota that accumulate contaminants from water only (species that actively ingest and assimilate organic material associated with sediment particles would be an exception). In addition, dissolved organic carbon (DOC) in water will tend to sorb hydrophobic organics to reduce the amount of contaminant available to an organism (Carter and Suffet 1982, Landrum *et al.* 1985, 1987, McCarthy and Jimenez 1985*a*, McCarthy *et al.* 1985, McCarthy and Black 1988, Servos and Muir 1989, Servos *et al.* 1989). Sorption to DOC also varies with composition of the DOM examined (Landrum *et al.* 1987). For more hydrophobic contaminants, the influence of organic carbon is greater, due to its increased affinity for the organic fraction of sediment particles. In addition, sorption to clay and other particulates and colloid formation can affect the proportions of bound and freely dissolved contaminants in the aquatic environment (Landrum and Robbins 1990).

The manner in which a contaminant partitions into various aqueous and nonaqueous phases can significantly affect its bioavailability. Partitioning between porewater and sediment particles has been described in two parts: one in a reversible pool and another in a resistant pool (Landrum and Robbins 1990). The fraction of contaminant that resides in each of these pools changes, depending upon the sorption duration, until equilibrium is reached, which may be lengthy (Karickhoff 1980, DiToro *et al.* 1982). Studies examining this phenomena with PAH congeners and polychlorinated biphenyls (PCBs) showed that equilibrium of contaminant disposition between porewater and sediment particles may take months to years to achieve (Karikhoff 1980, Coats and Elzerman 1986, Witkowski *et al.* 1988). During this equilibration phase, desorption can greatly affect the bioavailability of contaminant to benthic organisms. Long equilibration periods can also affect the

data obtained from bioassays which employ spiked sediment. The duration of the mixing process affected toxicity in *Daphnia* sp., where toxicity decreased as the mixing time increased (Stemmer *et al.* 1990). Bioavailability of PAH congeners was also shown to decrease upon spiked sediment aging from 3 to 60 days. However, in the same study, the bioavailability of some congeners increased while others remained constant from 60 to 150 days of sediment aging (Landrum *et al.* 1992*c*). Such conflicting results reflect both the properties of the chemicals and biological factors that act to alter the availability of contaminants.

Characteristics of the Biota

The ability to obtain and transport xenobiotics to the target receptor in biota is not only a function of physical, chemical, and environmental properties. Both the behavior of the organism and biotransformation of the contaminant can substantially affect bioavailability. Benthic organisms routinely used in bioassays with sediment-associated contaminants are those that are epifaunal (associated with the sediment and sediment-water interface) or infaunal (sediment burowers), although some water-column species such as fish and water fleas have been studied. Within the infaunal and epifaunal group, a myriad of feeding behaviors and life histories exist. Each of these species possess various behaviors that act to modify their exposure via manipulation of the environment. For example, the infaunal oligochaetes burrow through sediment and obtain food from ingested sediment particles. These organisms, appropriately named "conveyer belt deposit-feeders", ingest sediment over a range of depths, while they deposit gut contents on the sediment surface from posterior ends that protrude at the sediment-water interface (Karickhoff and Morris 1985, Robbins 1986). Bioturbation produced by these organisms disrupts any equilibrium established among sediment-associated contaminants, affecting bioavailability not only to the oligochaetes, but to all biota in the reworking zone.

In the case of epifaunal, surface deposit feeders, such as the clam *Macoma nasuta*, food is filtered primarily from the upper few millimeters of sediment. Such an organism would not be exposed to contaminants found at depth (Lee 1991). Given that contaminants are not uniformly distributed within sediment horizons, infaunal and epifaunal species would be exposed to substantially different concentrations of sediment-associated contaminants.

Not only is the mechanism of feeding important in determining availability of contaminants, but the type and availability of food is also important. For

example, the availability of contaminants can be extremely variable for discriminatory *vs.* nondiscriminatory feeders inhabiting the same space in the benthos. Midge larvae are relatively nondiscriminant feeders that eat small plants, animals, and detritus (Oliver 1971). Other benthic species (*Tubifex, Diporeia*) are quite selective in their feeding habits, and tend to select the smaller, organic rich particles of sediment for ingestion (McMurthy *et al.* 1983, Lydy and Landrum 1993). Since hydrophobic organics sorb mainly to the organic carbon fraction of sediment, discriminant feeders will be exposed to, and will therefore tend to accumulate more contaminants than nondiscriminant feeders, merely by their feeding preference alone.

An important factor to consider is the primary route of contaminant uptake to an organism living in the benthos. Recently, much discussion has focused upon the role that interstitial water plays in determining the uptake of hydrophobic contaminants from sediment. Interstitial water has been suggested as the dominant route of uptake for neutral organics with a log K_{ow} of less than 5 in most benthic and epibenthic species (Adams 1987). Other researchers have supported this theory, and have determined that uptake from porewater is the dominant uptake route for some neutral hydrophobic and heavy metal contaminants (Adams *et al.* 1984, Kemp and Swartz 1988). However, other studies reject the hypothesis of porewater as the primary route of exposure, and suggest that physiological characteristics and life history are major influencing factors (Boese *et al.* 1990, Winsor *et al.* 1990, Lee 1991). For example, the construction of tubes and burrows by various benthic organisms (*e.g., Chironomus* and *Hexagenia* spp.) and the method by which porewater is pumped through them must be considered when determining the bioavailability of contaminants. Rate of interstitial water ventilation as well as the type of burrow construction can either permit or hinder accumulation of contaminants that would otherwise be accumulated without the burrow.

Accumulation of hydrophobic contaminants is not only a function of their physical uptake, but the ability of the compounds to be extracted and assimilated by the organism once they are acquired. A contaminant may be efficiently taken in by a sediment ingester, but if the compound does not desorb off the sediment particles in the digestion process, they will merely pass through the gut, and accumulation of compound will not occur. Therefore, both the desorption and assimilation of contaminants across the gut membrane, dependent upon the species examined, will affect the bioavailability of the contaminant (Lee 1991). Other species differences, such as life cycle duration (*i.e.*, whether or not the organism will be able to reach

steady-state during its time in the benthos) and the optimal environment for growth (*e.g.*, pH, temperature, dissolved oxygen) will also determine bioavailability of the contaminants (Gerould *et al.* 1983, Fisher 1985, 1986, Fisher and Wadleigh 1986).

Another biotic factor to consider is the organism's ability to biotransform the contaminant. Although few benthic species have been studied to date, most invertebrates have the ability to transform metabolizable compounds, such as BaP via a cytochrome P-450 or P-450-like system (Leversee *et al.* 1982, Varanasi 1989). However, a few species such as the amphipod *Diporeia* spp. and the mussel *Mytilus edulis* have not been found to biotransform these compounds (James 1989). Consequently, any contaminant that can be biotransformed by an organism will significantly affect the availability and thus exposure to the site of action *in vivo*.

The same reasoning holds true for contaminants that are biotransformed by bacterial action before they are taken up by benthic organisms. Heterotrophic microorganisms, found in virtually all aquatic environments, acquire most of their energy by degrading dissolved particulate organic material (Conway 1982). They are capable of degrading both DOC as well as organic xenobiotics found in the aquatic environment. Thus, the various pathways of biotransformation - oxidation, reduction, and hydrolysis - used by microorganisms will ultimately affect the availability of the xenobiotic to other biota.

Indicator Species Used in Bioassays

Aquatic species possess a wide variety of feeding behaviors and life histories. The place in which a particular organism chooses to reside will greatly determine its exposure. A question too often overlooked in bioassay experimental designs is, "Where does the indicator species reside in its native habitat?" and "Does the experimental design consider the stress placed on the organism in a less than natural setting?" Unusual testing conditions placed upon the indicator species may greatly alter its response to a contaminant. In this regard, placing an infaunal species into an aqueous extract or porewater-only experimental system may create stress that can be expressed as either a more or less rapid accumulation of contaminant than would occur in a natural setting. This was shown in studies that subjected burrowing mayfly larvae to spent shale leachate in chambers with and without artificial burrows. Gill-beat frequency and mortality were increased and molting frequency was depressed in larvae without access to burrows, compared to those that had access to burrows (Henry *et al.* 1986). In another study with mayfly larvae, respiration rates were greater for those in chambers without added substrate

than in chambers with substrate (Ericksen 1968). The increased stress induced by the lack of substrate would likely contribute to increased uptake of xenobiotics in a contaminated system.

All of the previously described factors that influence bioavailability must be considered when choosing the proper indicator species for a bioassay. According to a recent EPA guidance document (U. S. EPA 1989*b*), "the choice of the test species can have a major influence on the success, ecological significance, and interpretability of a [bioassay]". Desirable qualities of a test species include ease of year-around collection, suitability to laboratory culture, adaptability to laboratory testing conditions, suitable size, tolerance to a wide variety of sediment types and salinities, ecological and economic importance, sensitivity to test materials, and ease of handling (ASTM 1992*a,b*, U. S. EPA 1989*b*). For sublethal toxicity testing, the animals should display highly visible sublethal effects, such as color changes, or changes in behavior. For accumulation bioassays, the species should be a sediment ingester and must be sufficiently resistant to contaminants in order to survive the duration of a test with a minimal level of mortality (U. S. EPA 1989*b*). Under these criteria, relatively short-lived species, such as mayfly or midge larvae would not be suitable for bioaccumulation assays that take longer than an average life cycle to complete.

A number of species have been recommended for use by testing agencies. ASTM presently recommends using the amphipods *Rhepoxynius abronius, Eohaustorius estuarius, E. sencillus,* and *E. washingtonianus* when conducting 10-day toxicity tests in marine and estuarine sediments (ASTM 1992*b*). The U. S. EPA presently recommends the marine species *Nereis diversicolor, Neanthes virens* (polychaetes), *Macoma nasuta, M. Balthica,* and *Yoldia limatula* (bivalves) for conducting bioaccumulation assays in contaminated sediments (U. S. EPA 1989*b*). For toxicity testing of freshwater sediments, ASTM recommends the amphipod *Hyalella azteca* and midge larvae *Chironomus tentans* and *C. riparius* (ASTM 1992*a*). In addition, the freshwater oligochaete *Lumbriculus variegatus* has been recommended by the U. S. EPA for bioaccumulation tests, due to its ease of laboratory culture, highly visible sublethal responses, and its feeding strategy (*L. variegatus* is one of the few freshwater infaunal sediment ingesters, Bailey and Liu 1980, Phipps *et al.* 1993). Each possess most of the attributes of a good bioassay species. However, since the bioavailability of contaminants can vary from species to species even after normalization to lipid and organic carbon content (McElroy and

Means 1988), it is important to compare a variety of test species when conducting toxicity and/or bioaccumulation bioassays (Giesy and Hoke 1989, U. S. EPA 1989*b*).

Bioassays have proved to be effective tools for assessing the toxicity of sediment-associated contaminants. However, unless consideration is made as to the various routes of uptake, feeding strategies, and behaviors inherent to the indicator species that influence the bioavailability of contaminants, data generated from such bioassays is of little worth. Research is needed that not only compares dose-response relationships among species and methodologies, but also examines the mechanisms and biochemistry of the toxicological process upon exposure. Such tasks will improve our understanding of the effects that sediment-associated contaminants place on the aquatic environment.

OBJECTIVES OF THE RESEARCH

The previous discussion has indicated that the use of whole sediments and extracts of sediments in bioassays as indicators of contamination have not been thoroughly investigated. Although toxicity bioassays have compared bulk sediment with that of porewater and other aqueous extracts, no such comparisons that use the kinetic approach have evaluated the differences in exposure and resulting effects among a variety of indicator species. Bioassays need to be conducted simultaneously in all three phases to adequately assess the exposure represented by whole sediment, porewater, and elutriates. Mechanisms that cause differences in accumulation among the exposure media then need to be examined to determine why these differences occur.

The approach that I have taken to address this issue is, first, to compare the routes of exposure in three species of benthic invertebrates by monitoring the accumulation of a number of hydrophobic organic contaminants possessing different physical-chemical properties from whole sediment, porewater, and elutriates. The three indicator species chosen include fourth instar midge larvae (*Chironomus riparius*), the aquatic oligochaete *Lumbriculus variegatus*, and the amphipod *Diporeia* spp. Each of these organisms possesses different feeding behaviors, physiologies, and life histories. From the data generated in these bioassays, differences in rates of contaminant uptake and therefore accumulation in biota will be compared via toxicokinetic modeling.

Mechanisms that affect differential accumulation of contaminants in the three species will next be investigated. These studies will determine the extent of biotransformation of contaminants and differences in lipid stores among the three indicator species selected. Differences in bioassay exposure conditions will be studied by comparing uptake of contaminants in organisms receiving additional food to those not receiving food during an assay. Exposure will also be investigated by determining assimilation efficiencies and differential feeding in one indicator species. Differences in physical and chemical parameters of the contaminants that affect bioaccumulation will also be examined via partitioning studies that determine the amount of bioavailable contaminant in porewater and elutriates.

The three major hypotheses for the research presented are as follows:

1. Exposure to hydrophobic organic contaminants in whole sediment will differ from porewater and elutriates produced from it.

2. Accumulation of sediment-associated contaminants will vary among species possessing different feeding behaviors and life histories.

3. The exposure represented by a number of hydrophobic organic contaminants in porewater and elutriates can be explained by comparing the proportion of contaminants bound to organic and inorganic material to that freely dissolved in the aqueous phase.

COMPARISON OF WHOLE SEDIMENT, ELUTRIATE, AND POREWATER EXPOSURES FOR USE IN ASSESSING SEDIMENT-ASSOCIATED ORGANIC CONTAMINANTS IN BIOACCUMULATION ASSAYS

Introduction

Contaminated sediments are a recognized problem in many coastal areas and are presently a significant regulatory issue. The bioassay approach has been used to assess the toxicity of such sediments (Giesy and Hoke 1989, Giesy *et al.* 1990, Green *et al.* 1993, Phipps *et al.* 1993). In the bioassay approach, organisms are exposed to whole sediments or aqueous extracts of whole sediments to evaluate and estimate contaminant concentrations that produce effects. Although whole sediment exposures may be the most realistic laboratory simulation of *in situ* exposure to indicator species, aqueous extracts of whole sediment, such as porewater and elutriates, have been used to circumvent problems that occur when whole sediments are diluted and in situations that require identification of soluble toxicants via toxicity identification evaluation (Samoiloff *et al.* 1983, Long *et al.* 1990, Schubauer-Berigan and Ankley 1991, Amato *et al.* 1992). To date, few studies have directly compared exposure of contaminants in whole sediments and their extracts by using the same species in simultaneous exposures. Of these studies, all comparisons of the various exposure types have only used toxicity as an endpoint (Ankley *et al.* 1991, Green *et al.* 1993). However, toxicity is strongly influenced by factors other than just the amount of toxicant available to the organism, such as oxygen concentration, habitat, temperature, and food. Thus, evaluating the bioaccumulation of toxicants by a variety of indicator species exposed to whole sediments and their aqueous extracts at non-lethal concentrations would increase our understanding of the basic processes involving exposure in these various representations of sediment, and would permit better interpretation of toxicity bioassays. Furthermore, bioaccumulation assays that compare the uptake of sediment-associated contaminants in whole sediments and their aqueous extracts would be useful in identifying the most appropriate organisms and conditions for evaluating sediment contamination.

In this study, a number of hydrophobic organic contaminants were used to compare temporal accumulation in three indicator species exposed to whole sediment, and porewater and elutriates obtained from the same contaminated sediment. The species, *Chironomus riparius* larvae, *Diporeia* spp., and *Lumbriculus variegatus*, differed in physiology and behavior, and represented a range of uptake

strategies (*e.g.*, feeding strategies and behavior) in freshwater benthic invertebrates. Midge larvae of *C. riparius* are considered nondiscriminant filter feeders (Walshe 1951, Coffman 1967, Oliver 1971). Larvae spend only a portion of their life cycle in the aquatic environment, with pupation and adult emergence occuring 15-21 days after hatching in room temperature laboratory cultures (ASTM 1992*a*). In contrast, both the amphipod *Diporeia* spp. and the oligochaete *L. variegatus* complete their life cycles in the benthic environment. *Diporeia* spp. are intermittent, selective feeders (McMurthy *et al.* 1983, Quigely *et al.* 1989, Lydy and Landrum 1993). Their high lipid content, reaching as much as 50% (dry weight basis, Gardner *et al.* 1985*a*) makes them an optimal species for studies involving the bioaccumulation of lipophilic organic contaminants. Conversely, *L. variegatus* is an infaunal sediment ingester (Bailey and Liu 1980, Phipps *et al.* 1993) that has demonstrated continuous feeding behavior in the laboratory. All three species have been used in sediment toxicity and bioaccumulation tests, and have the potential to be used as indicator species for assessing hazard of sediment-associated contaminants.

Two main questions were posed in this study: (1) Do porewater and/or elutriate extracts of whole sediment adequately represent whole sediment exposures, and (2) Does accumulation of hydrophobic organic contaminants depend on the indicator species used? To answer these questions, each of the three species was exposed to laboratory-dosed sediments and their generated porewater and elutriates containing sublethal concentrations of selected polycyclic aromatic hydrocarbons (PAH) and organochlorine insecticides. Accumulation of the contaminants was monitored to determine differences in the rates and routes of exposure among the three species. The specific objectives of the study were to measure accumulation kinetics for compounds possessing different physical and chemical properties, such as octanol:water partition coefficients, as a means of comparing bioavailability from the various representations of contaminated sediments. In addition, changes in the bioavailability of selected contaminants resulting from the length of sediment/contaminant contact time were detected by exposing organisms to the media after various intervals of sediment aging.

Materials and Methods

Chemicals

The compounds studied included ^{14}[C]-radiolabeled *trans*-chlordane (13.7 mCi/mmol, Velsicol Chemical Co., Memphis, TN), [^{14}C]-endrin (8.4 mCi/mmol, Sigma Chemical Company, St. Louis, MO), 4,4' [^{14}C]-DDT (11.8 mCi/mmol, Sigma

Chemical Company), [^{14}C]-benzo(*a*)pyrene (BaP, 16.2 mCi/mmol, Sigma Chemical Company), [^{3}H]-benzo(*a*)pyrene (40.0 Ci/mmol, Sigma Chemical Co.; 69.0 Ci/mmol, Amersham Ltd., Amersham, UK), [^{3}H]-chrysene (340.0 mCi/mmol, Chemsyn Science Laboratories, Lenexa, KS), and [^{3}H]-pyrene (25.2 Ci/mmol, Chemsyn Science Laboratories). All compounds were dissolved in an acetone carrier. Compound radiopurity was greater than 97% for all compounds prior to use, as determined by thin layer chromatography, using either benzene:ethyl acetate (3:1, v:v, endrin, DDT, and *trans*-chlordane) or hexane:benzene (8:2, v:v, BaP, chrysene, and pyrene) and liquid scintillation counting (LSC). All solvents were of HPLC grade. Analytical procedures were performed under gold fluorescent light (λ> 500 nm) to minimize the photodegradation of the PAH.

Sediment Dosing and Manipulation

Sediment was collected at a 45-m depth in Lake Michigan by Ponar grab approximately 8 km off the coast of Grand Haven, MI. The sediment was passed through a 1-mm screen sieve to remove debris and indigenous organisms. A sediment-water slurry was made by diluting wet sediment with Lake Michigan water in a 1:4 sediment to water ratio (w/v). Radiolabeled chemicals were added drop by drop in a minimal amount of acetone carrier (<1 ml) to the slurry while being stirred on a mechanical stirrer at room temperature for 4 h. Sediments for most assays were prepared with dual-labeled compounds in the following combinations: BaP/*trans*-chlordane, endrin/pyrene, DDT/chrysene, and *trans*-chlordane/pyrene, where one compound was ^{3}H-labeled and the other was ^{14}C-labeled. Two sediments were single-labeled with pyrene and BaP. After stirring, sediment slurries were left to settle at 4°C for 48 h. After the settling period, overlying water was decanted, and the sediment was washed with another volume of lake water. The mixture was again stirred by mechanical stirrer at room temperature for another 4 h, then left to settle for 48 to 96 h. The overlying water was again decanted, and portions of the sediment were either used to prepare elutriates or centrifuged to obtain porewater. In assays that used aged sediment, overlying water was decanted after the 96 h settling period, and was replaced with approximately 2L of fresh Lake Michigan water. The prepared sediment was then stored in the dark at 4°C.

Due to the potentially toxic concentrations of endrin to midge larvae used in the first endrin/pyrene assay, leftover sediment was diluted for the second endrin/pyrene assay. Sediment was diluted with two additional volumes of wet 45-m Lake Michigan sediment. Enough [^{3}H]-pyrene was added to bring the sediment pyrene

concentration to that of the previous assay, and the sediment was mixed for 4 h. The mixture was allowed to settle for 48 h prior to decanting overlying water and preparing elutriates, porewater, and whole sediment exposures for the second assay.

Elutriate Preparation

A 1:4 ratio (sediment:water, v:v) of prepared dosed sediment was used to prepare elutriates (U. S. EPA/COE 1977). The sediment/water mixtures were placed into 250-ml Teflon centrifuge bottles, placed on a laboratory rotator at 200 rpm for 30 minutes, then were allowed to settle for 1 h. The unsettled portions were decanted into clean Teflon centrifuge bottles and were centrifuged at 2000*g* at 10°C for 30 min. The elutriate fractions were decanted and stored for no longer than 24 hours at 10°C before use.

Porewater Preparation

Dosed prepared sediment was placed into 250-ml stainless steel centrifuge bottles and centrifuged at 4000*g* for 30 min at 10°C. Supernatant from the bottles was decanted into 250-ml Teflon centrifuge bottles and was spun at 2000*g* for 30 min at 10°C. The supernatant from all bottles was decanted and stored for no longer than 24 hours at 10°C before use.

Dosed Lake Water Preparation

All water used in the study was Lake Michigan surface water collected about 1 m below the surface and stored at 4°C until used. Quantities of lake water were filtered through 0.45-µm glass fiber filters to remove particles. Containers of lake water were placed on a laboratory stirrer upon the addition of radiolabeled compounds in acetone carrier. Compounds were added as either dual- or single-radiolabels with the same compound combinations as prepared dosed sediments. Mixtures were stirred at room temperature for at least 1 h and were stored overnight in the dark at 10°C before use.

Organisms

Diporeia spp.

Diporeia spp. were collected from surficial sediment at a water depth of 29 m in Lake Michigan in the fall, spring, and summer of 1991-1993. Organisms were screened from the sediments, transported to the laboratory in cool lake water, and kept in holding aquaria containing 3 to 4 cm lake sediment overlaid with 10 cm lake water at 4°C in the dark. Three days before the start of an assay, animals were removed from the sediment and placed into acclimating aquaria containing the same

substrate as the holding aquaria. *Diporeia* spp. were kept in the dark and were acclimated to 10°C by elevating the temperature by 2°C per day.

Chironomus riparius

Midge larvae were reared in the laboratory at ambient temperature (22-25°C) on a substrate of shredded brown paper towels and a diet of ground Tetramin® (TetraWerk, Germany) and Cerophyl® (AgriTech, Kansas City, MO), according to ASTM standards (ASTM 1992*a*). Fourth instar larvae were removed from the culture aquaria and placed in environmental chambers with the same photoperiod (18D:6N) and feeding schedule as in the original culture aquaria. Environmental chambers were equipped with yellow lights to minimize photodegradation of the contaminants. Larvae were acclimated to 10°C by lowering the temperature by not more than 2°C in a 24-h period.

Lumbriculus variegatus

L. variegatus were reared in the laboratory at room temperature on a substrate of shredded brown paper towels and a diet of U. S. Fish and Wildlife-certified trout chow, according to Phipps *et al.* (1993). Animals were removed from the culture aquaria and acclimated to 10°C on the same 18D:6N photo period as *C. riparius*.

Voucher material, representative of each of the three indicator species used in this study, was deposited in the arthropod collection at Clemson Univerisity, SC.

Experimental Procedure

Each assay exposed one of the indicator species to replicates of whole sediment, elutriate, and porewater for predetermined, timed intervals. In addition, replicates of dosed, filtered Lake Michigan water were used as reference aqueous exposures to establish baseline rates of uptake for species exposed to aqueous-phase contaminants. Three beakers of whole sediment, porewater, elutriate, and dosed lake water were initially set up for each sampling point in all assays. To more accurately compare contaminant uptake rates, all assays were conducted at 10° $\pm$ 2°C. Preliminary studies indicated relatively short time intervals were required to achieve steady-state contaminant body burdens for *C. riparius* and *L. variegatus*, but longer intervals were required for *Diporeia* spp.

Each whole sediment exposure consisted of 30 g prepared wet sediment overlaid with 20 ml Lake Michigan water in 50-ml beakers which was allowed to settle overnight before the addition of animals. Two animals were added to each beaker, then kept in an environmental chamber with a 18D:6N photo period when testing *C. riparius* and *L. variegatus*. Environmental chambers were equipped with

gold fluorescent lights (λ > 500 nm) to avoid PAH photodegradation. All beakers were loosely covered with plastic food wrap to minimize water evaporation during the assays. *Diporeia* spp. were kept in darkness throughout the exposures. Animals from three replicate beakers were removed after one-, six-, 24-, 48-, 72-, and 96-h. Additional replicates were prepared for seven-day *L. variegatus* exposures and seven-, 10-, and 14-d exposures for *Diporeia* spp. In some assays, only two replicate beakers were set up for porewater exposures. Elutriate, porewater, and dosed lake water exposures were set up similarly to whole sediment exposures, using 20 ml of aqueous material in each 50-ml beaker. Two animals were added to each beaker and placed in the same environmental chamber as whole sediment exposures.

Fifty-ml glass centrifuge tubes were prepared (one replicate for the first, middle, and last exposure period) containing 20 ml wet dosed sediment and 20 ml filtered lake water with no added organisms to act as controls for the whole sediment and porewater exposures. These tubes were placed in the same environmental chamber as the exposure beakers. At the completion of each timed interval, the control tube was centrifuged, and porewater and sediment were assayed for contaminant concentration and compared to concentrations in whole sediment and porewater exposure beakers.

Analyses

Concentration of Contaminants in Animals and Test Media

At the end of each exposure interval, individual animals were removed from the test media, rinsed in distilled water, blotted dry, weighed, and directly placed into xylene-based scintillation cocktail (3a70b; Research Products International, Inc., Mt. Prospect, IL). Organisms were left to stand for at least 24 h for direct extraction of the contaminant(s) prior to analysis of radioactivity on an LKB 1217 liquid scintillation counter.

Triplicate sediment samples were taken for dry-to-wet weight ratios and contaminant concentration. Aliquots of sediment were placed on foil and weighed, then dried at 90°C to constant weight for determination of moisture content. Contaminant concentration in the sediment samples was determined by placing approximately 100 mg wet sediment directly into scintillation cocktail and sonicating the sample for two min using a Tekmar high intensity ultrasonic processor (extraction recovery = 82 - 92% for BaP, and 93 - 99% for *trans*-chlordane; Lydy and Landrum 1993). Samples were left to stand in scintillation cocktail for at

least 48 h before determining activity. Amount of contaminant/g dry weight sediment was calculated against moisture content of the samples.

Aqueous samples (porewater, elutriate, and dosed lake water) for contaminant analysis were removed from exposure beakers in 2-ml aliquots, placed directly into 12 ml scintillation cocktail and radioactivity was determined via LSC. All samples analyzed via LSC were corrected for quench using the external standards ratio method after subtracting background.

Organic Carbon Determination

Total organic carbon (TOC) content of sediment samples was determined by drying the sediment to constant weight, treating with 1 *N* HCl to remove carbonates, redrying, and assaying organic carbon on a Perkin-Elmer 2400 CHN Elemental Analyzer. Samples were measured against an acetanilide standard. Triplicate analyses yielded differences of 0 - 3%.

Replicate samples of elutriate, porewater, and dosed lakewater exposures were obtained for TOC determination at the beginning, middle, and end of each assay. Unfiltered, 2-ml samples were placed into pre-combusted ampules and frozen for later TOC determination. TOC was performed on an Oceanography International carbon analyzer after persulfate digestion (Golterman *et al.* 1978). Triplicate analyses yielded differences of 1 - 10%. In some aqueous exposures, TOC decreased significantly and linearly with time. In these cases, TOC concentration for individual exposure intervals was extrapolated from the regression produced from TOC analyzed at the beginning, middle, and end of the assay.

Biotransformation Studies

Biotransformation of pyrene, BaP, and *trans*-chlordane was examined in each of the indicator species after exposure in dosed lake water or whole sediment for 2 to 14 d. Ten to 20 animals were removed from the exposure media, rinsed in distilled water, blotted dry, and placed in a Ten Brock tissue homogenizer. Samples were acidified with 5 drops concentrated HCl and extracted with 2 X 5 ml ethylacetate : acetone (4:1 v:v) and 1 X 5 ml benzene. The extracts were combined, dried over anhydrous Na_2SO_4, and the volume was reduced to approximately 100 µl on a Zymark Turbo-Vap® evaporator. The extracts were analyzed by thin layer chromatography (TLC; Leversee *et al.* 1982). Extracts analyzed for *trans*-chlordane and pyrene along with standard parent compounds were developed in pentane : diethylether (9:1 v:v). Developed plates were divided into 2-cm sections, scraped from the TLC plates into scintillation cocktail, and activity was determined. Percent

parent compound was determined by the amount of extract corresponding to the Rf of the parent compound. Analysis of BaP was determined by 2-dimensional TLC with pentane:diethylether (9:1) and toluene:methylenechloride:methanol (25:10:1). The spots were identified by UV light and quantified by LSC. Standards of BaP metabolites for TLC chromatography were obtained from the National Cancer Institutes' Standard Chemical Reference Repository.

Contaminant purity of a dual-labeled *trans*-chlordane/BaP-spiked sediment and a single-labeled pyrene-dosed sediment was analyzed after standing in the dark at 4°C for 6.5 months (*trans*-chlordane/BaP), and 2 months (pyrene). Replicates of approximately 3 g wet sediment were placed into 50-ml centrifuge tubes, and the samples were acidified with 10 drops of concentrated HCl. Samples were extracted with 2 X 30 ml ethyl acetate:acetone (4:1, v:v), followed by a final extraction with 30 ml benzene. Samples were pulse sonicated for 3 min after each extraction, then centrifuged. The supernatant was combined and dried over anhydrous Na_2SO_4, and the volume reduced by rotary flash evaporation followed by evaporation under a stream of nitrogen to approximately 500 µl. The extracts were analyzed by TLC using hexane:benzene (8:2, v:v, BaP) or pentane:diethylether (9:1, v:v, *trans*-chlordane) and LSC as described above.

Lipid Analyses

Lipid content of individual *Diporeia* spp. and groups of two *C. riparius* and *L. variegatus* was analyzed after exposure of three- to 14-day intervals in dosed lake water, porewater, elutriate, or whole sediment. To accurately determine changes in lipid content during exposures, lipid analysis was also performed on animals directly obtained from culture aquaria or, for *Diporeia* spp., holding tanks. A micro-gravimetric procedure with a chloroform/methanol extraction was used for all lipid determinations (Gardner *et al.* 1985*b*).

Comparison Evaluation

To compare the rate of contaminant uptake among whole sediment, elutriate, porewater, and dosed lake water exposures, the concentration of contaminant readily available to the organism must be known. For aqueous fractions, this "freely dissolved" fraction has been accurately measured, using various partitioning methods, such as equilibrium dialysis (Carter and Suffet 1982), C-18 reverse phase separation (Landrum *et al.* 1984, Eadie *et al.* 1990), or water solubility enhancement (Chiou *et al.* 1986). However, no chemical measures currently exist to predict bioavailability of sediment-bound fractions. Consequently, no accurate comparison

regarding bioavailability of contaminants in whole sediment and aqueous extracts can be made by simply determining total contaminant concentration in the exposure media. However, hydrophobic organic contaminants are known to partition strongly to organic carbon (Knezovitch *et al.* 1987, Landrum and Robbins 1990). If one assumes that the contaminants used in this study partition to organic carbon similarly in whole sediment, elutriate, and porewater exposures, then uptake and accumulation comparisons can be made among the different exposure media, based on the available form of the contaminant from the organic carbon fraction alone. Therefore, by normalizing contaminant concentration in the various media to the amount of total organic carbon in the exposure system, we may estimate the relative bioavailability among whole sediment and aqueous extracts of whole sediment.

Carbon-normalized concentrations of contaminants in aqueous media (porewater, elutriate, and dosed lake water) were calculated according to the equation

$$\frac{\text{aqueous concentration (ng/ml)}}{\text{total organic carbon concentration in aqueous media (ng/ml)}} \cdot$$

Carbon-normalized concentrations of contaminants in whole sediment were calculated according to the equation

$$\frac{\text{wet weight sediment concentration (ng/ml)}}{\text{total organic carbon concentration in wet sediment (ng/ml)}} \cdot$$

Wet weight sediment concentrations were based on the average dry:wet ratios obtained from individual assays that ranged from 0.59 to 0.69.

The units used to compare bioaccumulation in aqueous fractions were defined as

$$\frac{\mu\text{g contaminant/g wet weight animal}}{\text{ng/ml contaminant in media} \cdot \text{ng/ml total organic carbon in media}^{-1}} \cdot$$

Kinetics Models

Uptake rate coefficients were determined using either one- or two-compartment models. The one-compartment model is described as

$$C_a = K_{uoc} C_m t$$

where

C_a = the concentration in the animal (ng $\cdot g^{-1}$),

K_{uoc} = the conditional uptake rate coefficient $\left(\frac{\mu g \text{ organic carbon cleared of contaminant}}{\text{gram organism wet weight/hour}}\right)$,

C_m = organic carbon normalized concentration of contaminant in the media (units as above), and

t = time.

This model assumes that the concentration of contaminant in the media remains constant over the interval of the assay. For whole sediment exposures, contaminant concentration, as well as total organic carbon concentration did not change, and the one-compartment model was used. However, both the total organic carbon content and the total concentration of contaminant changed in a linear fashion over time in most aqueous exposures. In these cases, it was necessary to incorporate the media concentration changes into a two compartment model

$$C_a = K_{uoc} [C_{w1} (t - \frac{s}{2} t^2)]$$

where

C_{w1} = organic carbon normalized concentration of contaminant in the media at time = 1 h (units as above), and

s = slope of the regression line of organic carbon normalized media concentration with time.

Both the one- and two-compartment models assume that elimination is unimportant over the time period for which the uptake rate coefficient is calculated, and any biotransformation and/or elimination of contaminants will be sufficiently diluted by the exposure media as to not result in a significant re-uptake of metabolite. In studies where a significant amount of contaminant was biotransformed in a short period of time, estimates of re-uptake of metabolites were determined and found to be less than 2% of the total body burden.

Statistics

Two-factor analysis of variance (ANOVA) was used to test for significant differences in contaminant accumulation among media (whole sediment, elutriate, and porewater exposures) and exposure intervals after log-transforming the data (SAS Institute 1985). Student's *t*-tests were used to test for overall significant differences in contaminant accumulation among whole sediment and aqueous phases at individual exposure intervals using Data Desk ® Professional software (Data Description, Inc. 1985). Differences were considered significant between the test categories at the 0.05 probability level. Rate coefficients and linear regression

analyses were calculated with the regression packages used in SAS® and Microsoft ® Excel (Microsoft Corp. 1991).

Results

Contaminant accumulation between whole sediment and aqueous exposures differed significantly for at least one sampling time in all assays. Representative assays showed that the ratios of mean elutriate accumulation to mean whole sediment accumulation and the ratios of mean porewater accumulation to mean whole sediment accumulation differed among sampling times, and generally displayed upward or downward trends throughout the sampling times (Figures 2.1-2.6; a complete summary of accumulation values for all bioassays can be found in Tables A-I through A-XII). Two-factor ANOVA showed contaminant accumulation to be significantly affected by the exposure media as well as the length of exposure for all assays except when *Diporeia* spp. were exposed to chrysene. Of the contaminants studied, aqueous fractions containing pyrene appeared to most closely represent exposure to whole sediment (Figure 2.1), while aqueous fractions containing *trans*-chlordane represented a significantly lower exposure than that represented by whole sediment (Figure 2.6). However, both greater and lower accumulation from elutriate and porewater versus whole sediment exposures were observed and were dependent upon the sampling times as well as the indicator species used.

Pyrene Accumulation

Elutriates most closely reflected the exposure of pyrene-dosed sediment, with mean elutriate:sediment ratios of approximately one for all indicator species, except *L. variegatus* at 7 days (Figure 2.1*a*). For the representative assay, mean pyrene accumulation was only significantly lower in elutriates compared to whole sediment at one, 72, and 96 hours (*C. riparius*), and six hours (*Diporeia* spp. and *L. variegatus*; Figure 2.1*a*). Pyrene accumulation from elutriates was greater than that from whole sediment in *L. variegatus* for all exposures observed after 48 hours (Figure 2.1*a*).

Accumulation of pyrene from porewater exposures was generally lower than accumulation from whole sediment (Figure 2.1*b*). No significant differences between pyrene accumulation from porewater and whole sediments were observed at one, 24, 96, and 240 hours (*Diporeia* spp.), one, 72, and 96 hours (*C. riparius*), and one week (*L. variegatus*). In some cases, a wide variation in pyrene accumulation among animal replicates sampled after the same exposure intervals was seen (see Tables A-II, A-VI, and A-XI). Uptake rate coefficients consistantly demonstrated less pyrene uptake from either aqueous extract than from whole sediment (Tables 2.1 and 2.2).

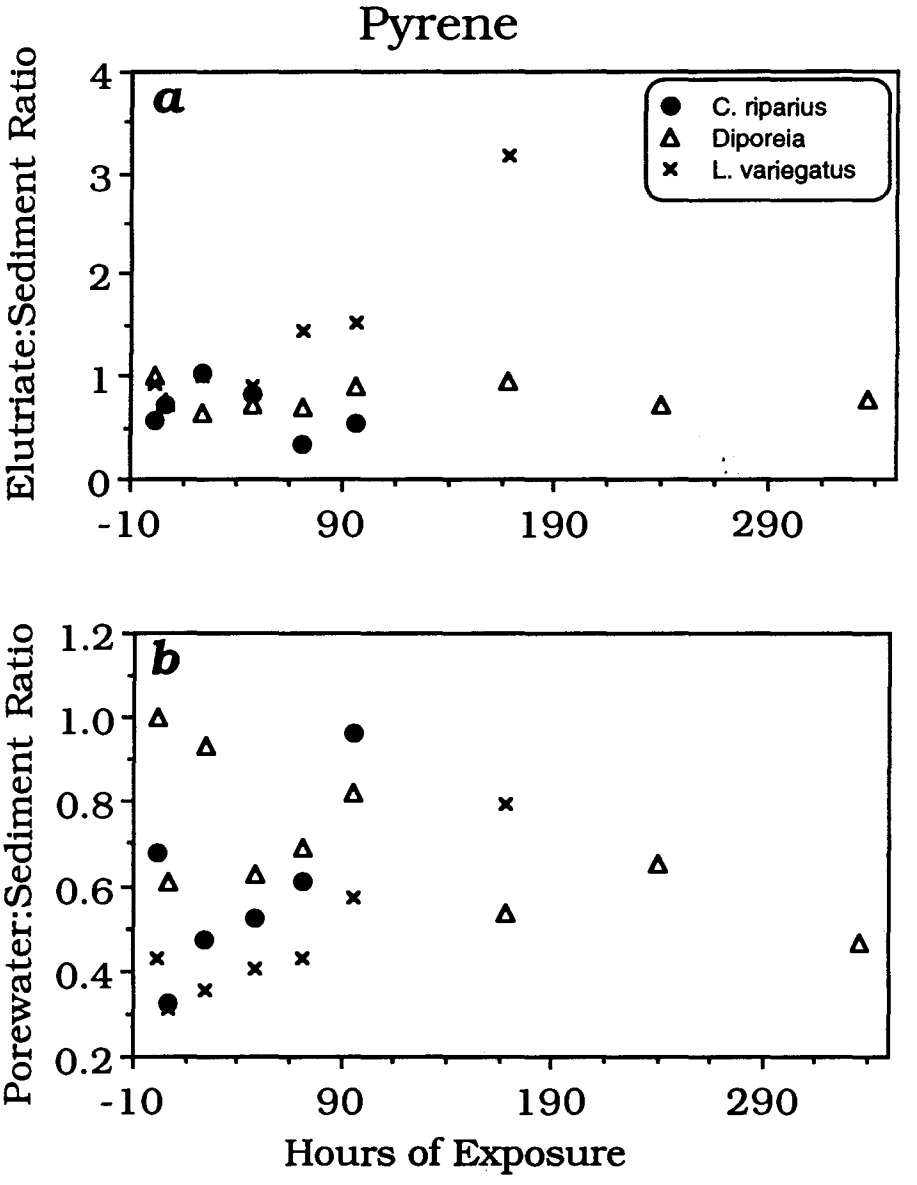

Figure 2.1. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of pyrene from one to 96 hours (*C. riparius*), one to 168 hours (*L. variegatus*), and one to 336 hours (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

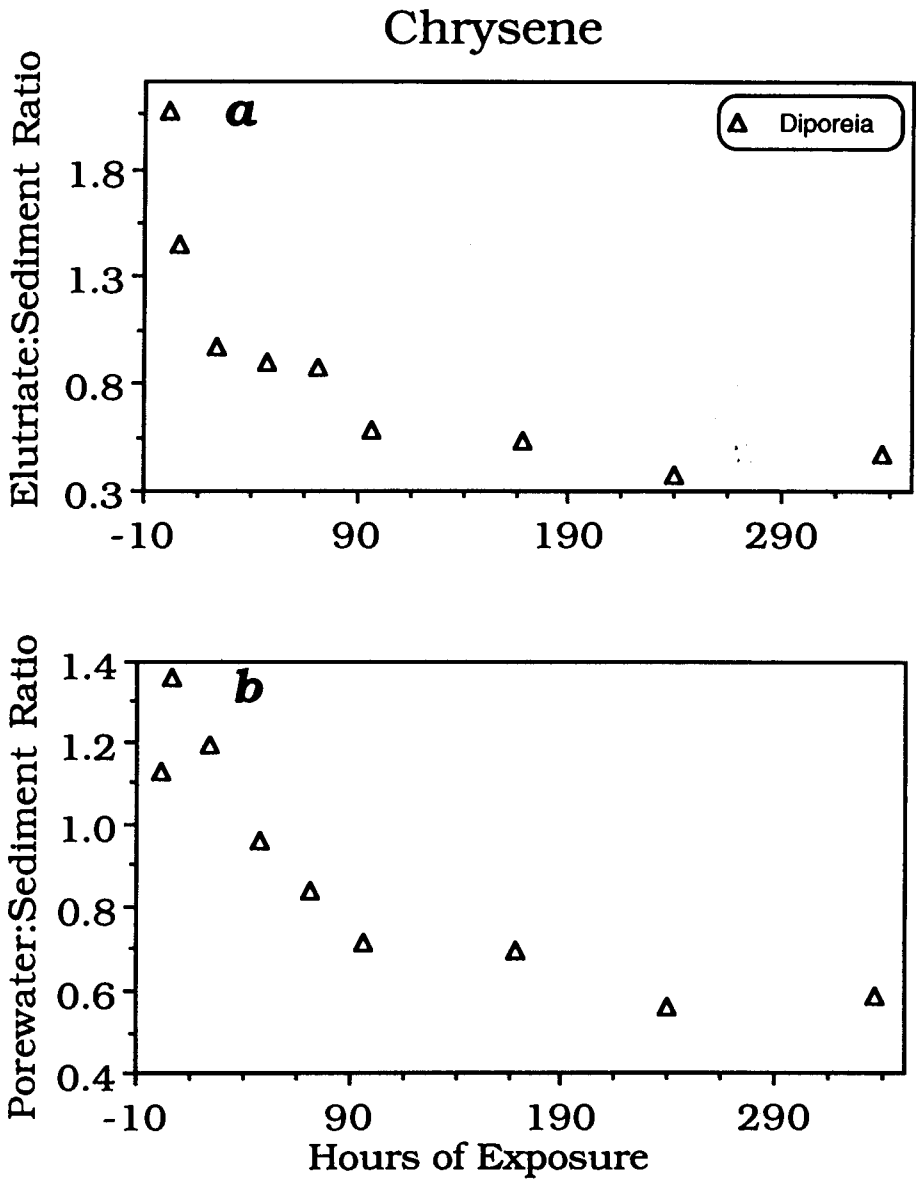

Figure 2.2. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of chrysene from one to 336 hours (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

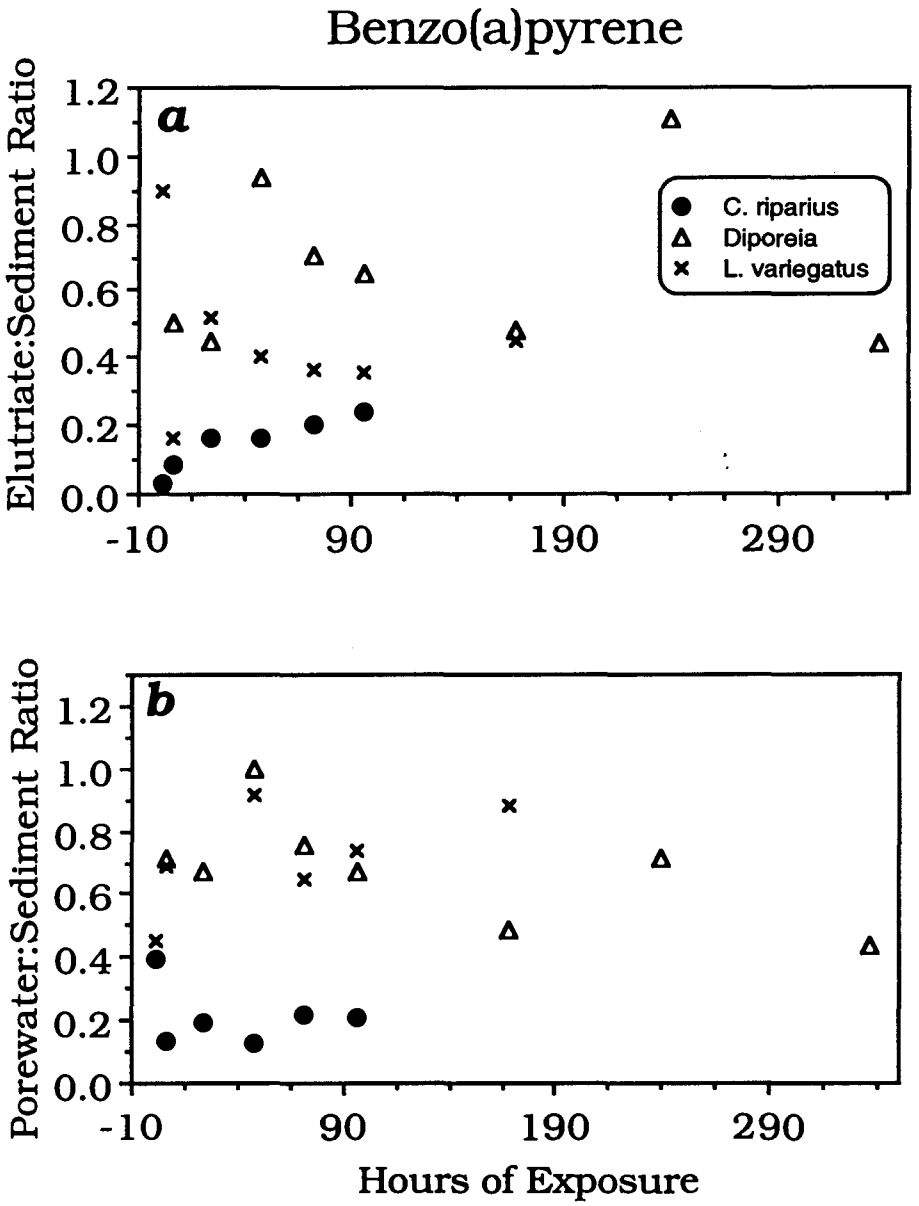

Figure 2.3. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of benzo(*a*)pyrene from one to 96 hours (*C. riparius*), one to 168 hours (*L. variegatus*), and one to 336 hours (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

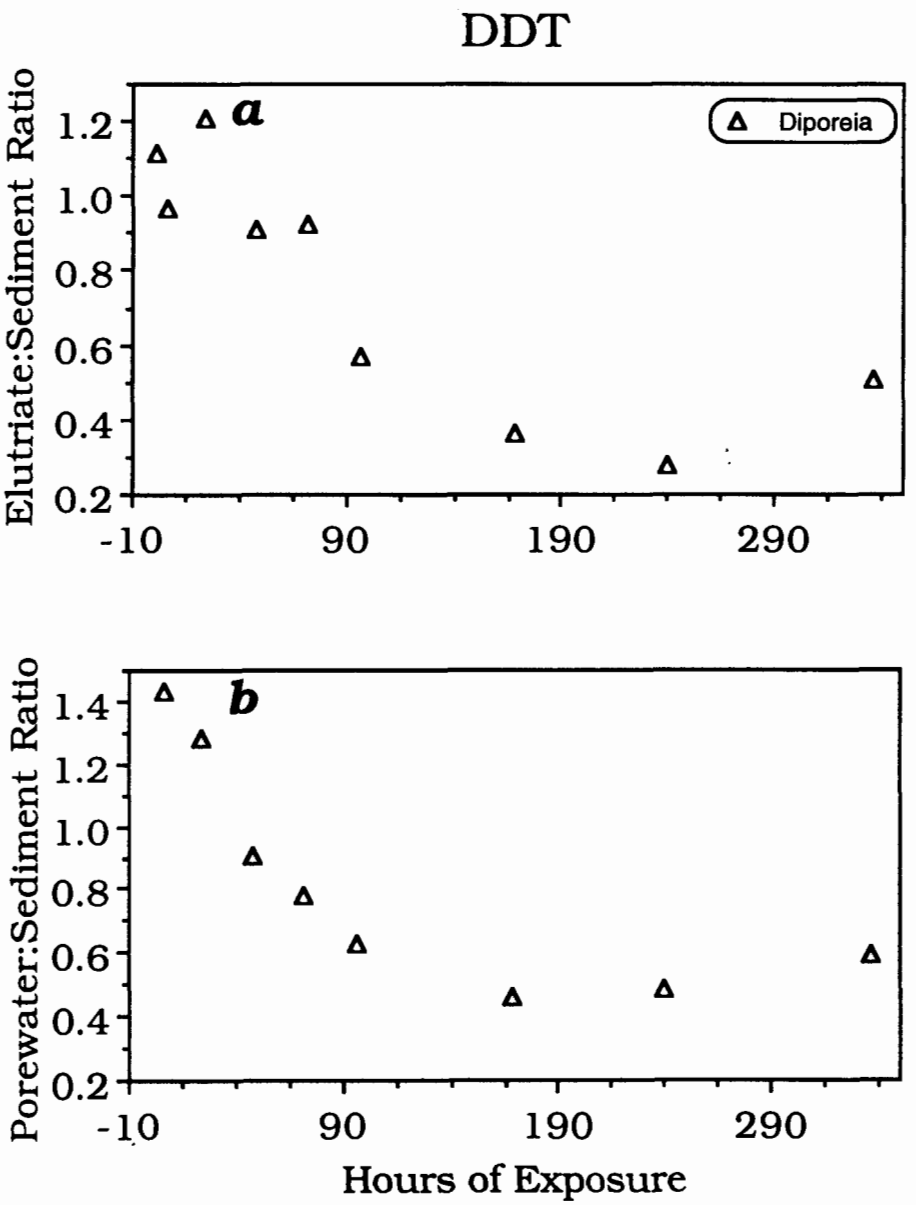

Figure 2.4. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of DDT from one to 336 hours (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

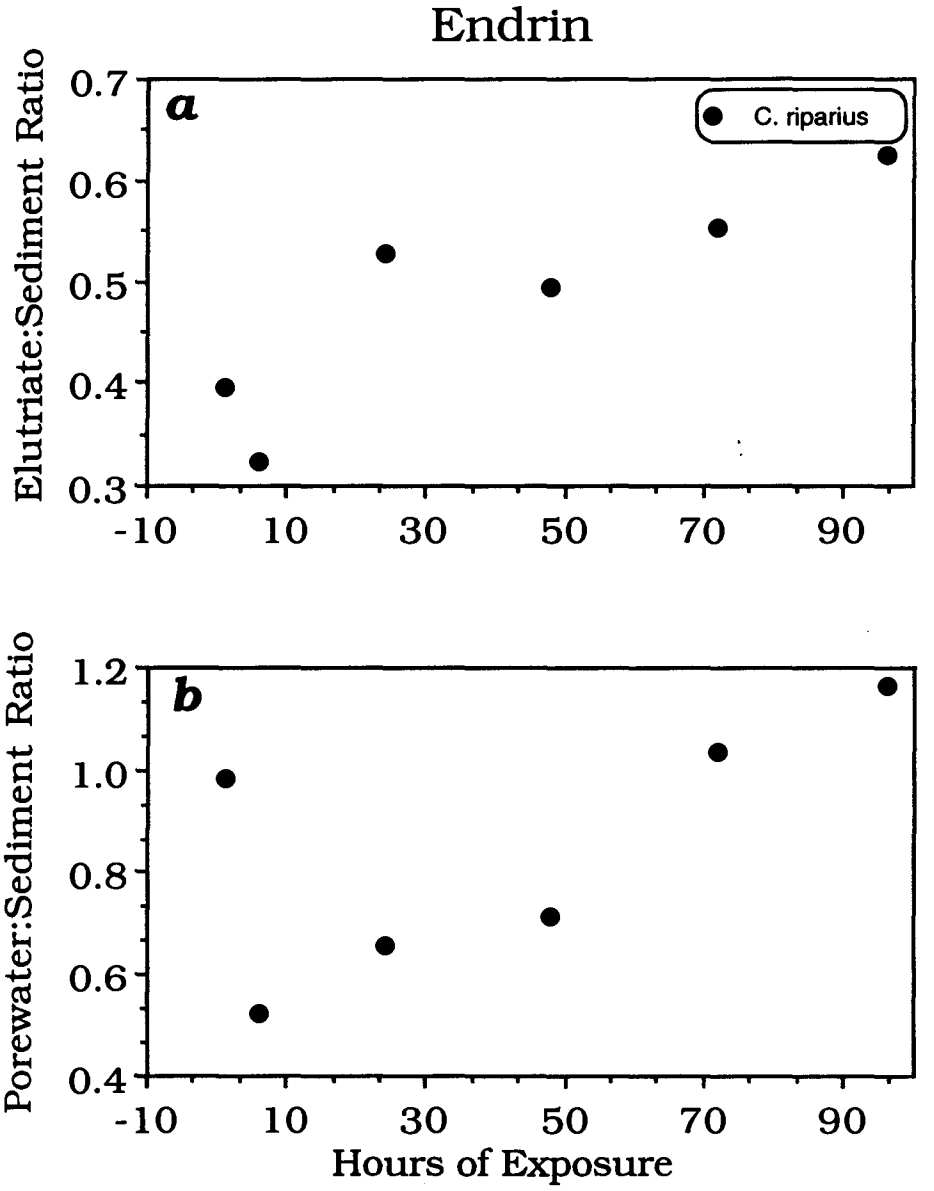

Figure 2.5. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of endrin from one to 96 hours (*C. riparius*). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

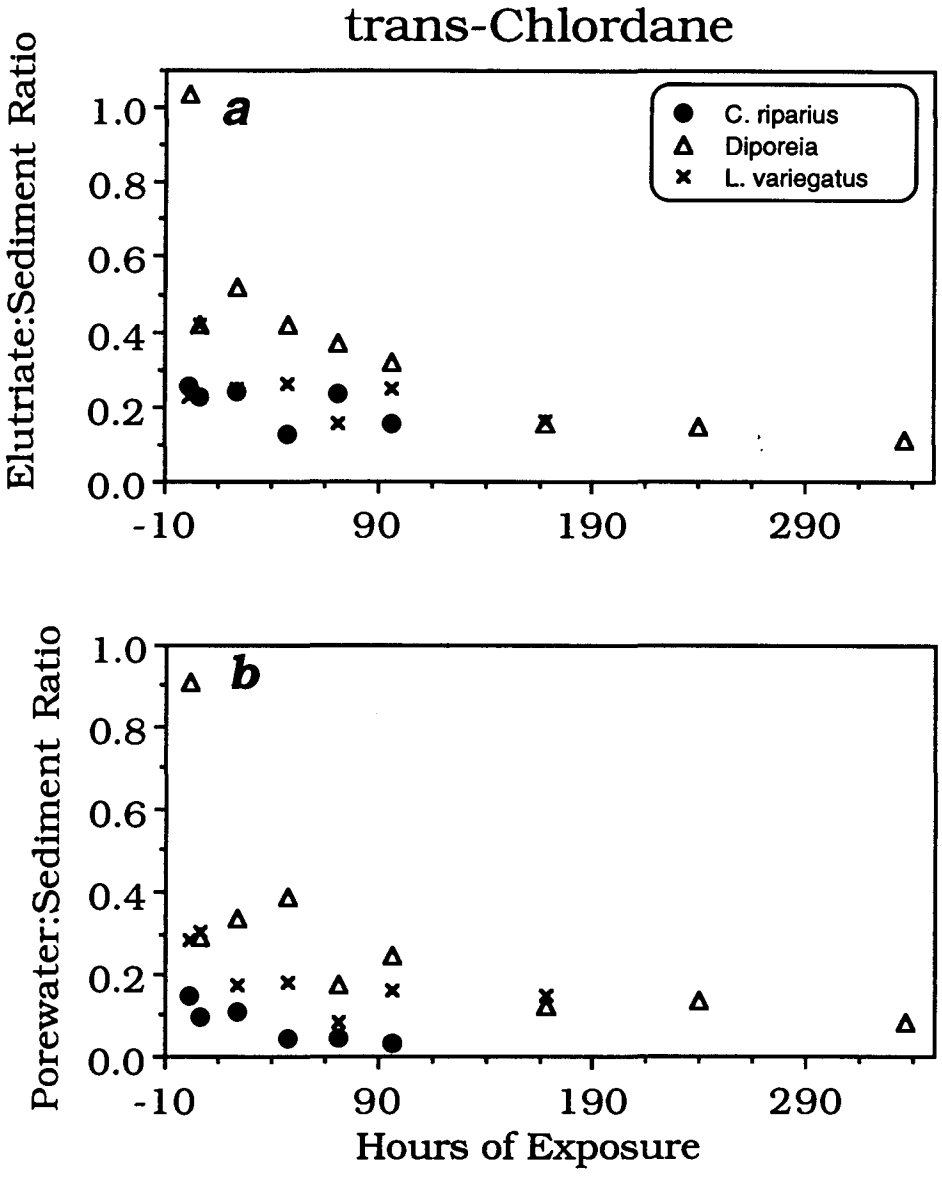

Figure 2.6. Ratio of (*a*) elutriate:sediment and (*b*) porewater:sediment bioaccumulation of *trans*-chlordane from one to 96 hours (*C. riparius*), one to 168 hours (*L. variegatus*), and one to 336 hours (*Diporeia* spp.). Each point represents ratios calculated from the mean accumulation of three to six animals from separate elutriate, porewater, and whole sediment exposures.

TABLE 2.1

K_{uoc} VALUES IN *Diporeia* SPP., *C. riparius*, AND *L. variegatus* FOR CONTAMINANTS IN WHOLE SEDIMENT AND ELUTRIATE EXPOSURES

Compound	Whole Sediment			Elutriates		
	Diporeia	*C. rip.*	*L. var.*	*Diporeia*	*C. rip.*	*L. var.*
trans-Chlordane						
Assay 1	246.7[a]	358.1	355.6	58.7	66.6	117.9
	(18.0)[b]	(25.0)	(41.6)	(3.9)	(5.7)	(3.9)
Assay 2	216.8	nd[c]	356.6	46.9	nd	72.8
	(12.6)		(29.0)	(3.2)		(4.7)
Assay 3	252.7	nd		55.1	nd	nd
	(16.0)			(4.3)		
BaP						
Assay 1	15.3	15.7	53.5	8.7	2.7	8.1
	(2.6)	(1.4)	(5.0)	(0.2)	(0.3)	(0.7)
Assay 2	19.6	15.0	nd	3.9	4.4	nd
	(1.5)	(1.4)		(0.2)	(0.2)	
Assay 3	11.0	nd	nd	4.3	nd	nd
	(1.4)			(0.2)		
Pyrene						
Assay 1	319.1	165.9	185.2	134.1	61.4	95.5
	(28.0)	(9.4)	(12.8)	(12.6)	(3.6)	(4.8)
Assay 2	176.0	nd	153.2	158.2	nd	82.0
	(11.7)		(23.5)	(14.1)		(4.8)

[a] units for K_{uoc} are: $\frac{\mu g \text{ organic carbon cleared}}{\text{gram organism/hour}}$

[b] $\pm$ standard error

[c] nd = not determined

TABLE 2.2

K_{uoc}VALUES IN *Diporeia* SPP., *C. riparius*, AND *L. variegatus* FOR CONTAMINANTS IN POREWATER AND REFERENCE LAKE MICHIGAN WATER EXPOSURES

Compound	Porewater			Lake Michigan Water		
	Diporeia	*C. rip.*	*L. var.*	*Diporeia*	*C. rip.*	*L. var.*
trans-Chlordane						
Assay 1	51.0[a]	80.1	57.5	236.0	30.6	159.8
	(3.5)[b]	(14.8)	(3.6)	(2.7)	(3.3)	(16.1)
Assay 2	70.9	nd[c]	63.3	224.8	nd	159.6
	(4.1)		(4.5)	(14.9)		(19.5)
Assay 3	74.7	nd	nd	nd	nd	nd
	(4.5)					
BaP						
Assay 1	12.5	3.2	30.4	242.9	41.3	176.8
	(0.6)	(0.2)	(2.0)	(50.0)	(5.4)	(9.2)
Assay 2	6.9	7.6	nd	184.7	24.2	nd
	(0.4)	(0.6)		(16.3)	(1.6)	
Assay 3	14.2	nd	nd	nd	nd	nd
	(1.0)					
Pyrene						
Assay 1	250.0	85.9	90.7	231.7	40.1	181.0
	(21.9)	(7.1)	(4.9)	(38.8)	(6.3)	(9.9)
Assay 2	133.3	nd	55.0	276.9		159.9
	(10.1)		(2.9)	(20.9)		(19.5)

[a]units for K_{uoc} are: $\frac{\mu g \text{ organic carbon cleared}}{\text{gram organism/hour}}$

[b]$\pm$ standard error

[c]nd = not determined

Pyrene uptake was greatly enhanced when it was used in a dual-labeled assay with [^{14}C]-endrin at non-toxic concentrations (*e.g.*, no mortality or apparent behavioral changes). Pyrene K_{uoc} values for *C. riparius* exposed to dual-labeled media were 936.9 ± 164 µg organic carbon cleared · g^{-1} · h^{-1} (whole sediment), 142.0 ± 23 (elutriate), and 346.2 ± 35 (porewater), compared to K_{uoc} values of 165.9 ± 9 (whole sediment), 61.4 ± 4 (elutriate), and 85.9 ± 7 (porewater) found for single-labeled exposures.

Chrysene Accumulation

A single assay was performed with *Diporeia* spp. exposed to chrysene. No significant accumulation differences were seen among exposure media (ANOVA: $F = 0.39$; $df = 2,99$; $P = 0.681$), although accumulation was significantly different among exposure intervals. Using Student's *t*-tests, accumulation of chrysene was significantly greater in elutriate exposures than whole sediment after one hour, but was not significantly different between six- and 72-h periods. After 72 hours, accumulation of chrysene from elutriate was significantly lower than from whole sediment, and the elutriate:sediment accumulations followed a downward curve over time (Figure 2.2*a*). A similar curve was observed for porewater:sediment accumulation ratios (Figure 2.2*b*). However, porewater accumulation was only significantly lower than whole sediment chrysene accumulation after 96 h of exposure. Uptake rate coefficients for *Diporeia* spp. exposed to chrysene were highest in porewater (244.3 µg organic carbon cleared · g^{-1} · hr^{-1}) and lowest in elutriate (55.2 µg organic carbon cleared · g^{-1} · hr^{-1}; Table 2.3).

Benzo(*a*)pyrene Accumulation

Accumulation differences between BaP-dosed whole sediment and aqueous extracts appeared to be a function of the species examined. *C. riparius* larvae accumulated considerably less BaP in elutriate and porewater than from whole sediment over all exposure intervals (Figure 2.3*a,b*). In most cases, accumulation in midge larvae from elutriates was comparable to that from porewater. BaP accumulation in *Diporeia* spp. was also generally lower in elutriate and porewater than in whole sediment. However, for a few sampling times (48 and 240 hours in elutriate, Figure 2.3*a*; six, 48, and 96 hours in porewater, Figure 2.3*b*), mean accumulation in *Diporeia* spp. was not different from that in whole sediment. Accumulation of BaP in *L. variegatus* was generally lower in aqueous extracts compared to whole sediment, and *L. variegatus* accumulation from whole sediment was greater than elutriate accumulation by a factor of approximately six after six

hours (Figure 2.3*a*). For *L. variegatus* exposures in porewater, accumulation was not different than accumulation from sediment, except at 96 hours, where porewater accumulation was significantly lower than that from sediment.

TABLE 2.3

K_{uoc} VALUES IN *Diporeia* SPP. FOR DDT AND CHRYSENE IN WHOLE SEDIMENT, ELUTRIATE, AND POREWATER EXPOSURES

Compound	Whole Sediment	Elutriate	Porewater
DDT			
Assay 1	91.0[a]	120.5	205.7
	(5)[b]	(7)	(43)
Chrysene			
Assay 2	128.5	55.2	244.3
	(7)	(13)	(33)

[a]units for K_{uoc} are: $\frac{\mu g \text{ organic carbon cleared}}{\text{gram organism/hour}}$

[b]$\pm$ 1 standard error

Uptake rate coefficients for BaP were the lowest of any for the contaminants studied and ranged from 11.0 (*Diporeia* spp.) to 53.5 (*L. variegatus*) in sediment, and from 2.7 (*C. riparius*) to 30.4 (*L. variegatus*) in aqueous extracts (Tables 2.1 and 2.2). Of the three species examined, *L. variegatus* consistantly cleared BaP from the organic carbon most rapidly, while *C. riparius* and *Diporeia* spp. tended to clear approximately the same amounts of contaminant from organic carbon per time.

DDT Accumulation

The accumulation of [^{14}C]-DDT by *Diporeia* spp. was monitored along with [^{3}H]-chrysene in a single assay. Accumulation of DDT was not significantly different between sediment and elutriate through 96 h (Table A-VIII). Porewater/sediment accumulation comparisons appeared similar to elutriate/sediment comparisons, except that mean porewater accumulation was significantly greater than from whole sediment accumulation at the first sampling time (1 hour). Both elutriate and porewater DDT accumulations were significantly lower than whole sediment

accumulation after 96-h exposures, and showed a downward trend (decreasing elutriate:sediment and porewater:sediment accumulation ratios) from 24 to 240 h (Figure 2.4*a,b*). Uptake rate coefficients for DDT in *Diporeia* spp. were highest in elutriate and porewater exposures, where sediment K_{uoc} was less than half that obtained for porewater (Table 2.3). No differences in DDT accumulation between elutriate and whole sediment exposures and between porewater and whole sediment exposures were observed for most of the early sampling times (Figure 2.4*a,b*).

Endrin Accumulation

The first endrin assay conducted with *C. riparius* produced significant mortality. Survival of *C. riparius* was 100% in whole sediment exposures up to 72 h, but dropped to 50% at 96 h. Aqueous exposures appeared even more toxic, with 100% survival to 24 h in both elutriate and porewater, declining to 66% at 48 h, 33 to 66% at 72 h (elutriate and porewater, respectively), and 50% to 83% at 96 h (porewater and elutriate, respectively). In a second assay, unused sediment from the first assay was diluted with clean 45-m Lake Michigan sediment, so that the endrin concentration dropped from 1.5 µg/g dry weight (326 µg/g organic carbon) to 0.5 µg/g dry weight (109 µg/g organic carbon). Survival of *C. riparius* was 100% in Assay 2.

In Assay 2, mean elutriate:sediment accumulation ratios were all less than one (Figure 2.5*a*). Mean porewater accumulation was significantly less than whole sediment accumulation at six-, 24-, and 48-h exposure periods (Figure 2.5*b*). An upward trend in elutriate:sediment and porewater:sediment accumulation ratios after six h was apparent (Figure 2.5*a,b*). Uptake rate coefficients were substantially lower in Assay 2 than in Assay 1 (Table 2.4), where the calculated K_{uoc} in whole sediment was over nine times greater in the first assay than in the second assay. Uptake rate coefficients from Assay 1 data in elutriate and porewater were much lower than those calculated in whole sediment (Table 2.4). Elutriate and porewater uptake rate coefficients in Assay 2 were also reduced from those observed in Assay 1, but were comparable to that of the K_{uoc} calculated for whole sediment (Table 2.4).

trans-Chlordane Accumulation

Accumulation of *trans*-chlordane was generally greater from whole sediment exposures than from the aqueous extracts for all of the species tested. *Diporeia* spp. accumulation from the aqueous extracts was comparable to whole sediment only at the first sampling time (Figure 2.6*a,b*). However, accumulation of *trans*-chlordane was greater in sediment than in either porewater or elutriate at one h for another assay that used *Diporeia* spp. (Table A-V). Mean elutriate:sediment and

porewater:sediment accumulation ratios were all substantially less than one for *L. variegatus* and *C. riparius* (Figure 2.6*a,b*). These differences were reflected in K_{uoc} values that were highest in whole sediment exposures for all three species, ranging from 46.9 - 117.9 in elutriate and porewater to 216.8 - 358.1 in whole sediment (Tables 2.1 and 2.2). Accumulation of *trans*-chlordane in both *C. riparius* and *Diporeia* spp. was comparable in elutriate and porewater. Accumulation from elutriate was generally greater than that from porewater in *L. variegatus* over the course of the assays, where mean elutriate accumulation was approximately twice that of porewater accumulation in one *L. variegatus* assay (Table A-X).

TABLE 2.4

K_{uoc} VALUES IN *C. riparius* FOR CONTAMINANTS IN ENDRIN-DOSED SEDIMENT, ELUTRIATE, POREWATER, AND REFERENCE LAKE MICHIGAN WATER EXPOSURES[a]

	Whole Sediment	Elutriate	Porewater	Lake Michigan Water
Assay 1	1,726.9[b]	511.4	298.3	40.5
	(308)[c]	(47)	(93)	(2)
Assay 2	189.2	113.8	128.2	nd[d]
	(21)	(12)	(9)	

[a]Endrin-dosed sediment at 1,477 ng/g dry weight in Assay 1 was diluted with clean sediment to a concentration of 527 ng/g dry weight in Assay 2.

[b]units for K_{uoc} are: $\frac{\mu g \text{ organic carbon cleared}}{\text{gram organism/hour}}$

[c]$\pm$ 1 standard error

[d]not determined

Dosed Lake Water Accumulation

Accumulation of *trans*-chlordane, BaP, and pyrene from dosed lake water exposures was generally greater than accumulation from aqueous and whole sediment exposures, but varied greatly among species and among sampling times. Uptake rate coefficients for BaP were higher in dosed lake water exposures than in

any of the test phases for all three species, but were either greater, less, or comparable to K_{uoc}s calculated for *trans*-chlordane and pyrene in the various media (Tables 2.1 and 2.2). Uptake rates from dosed lake water exposures replicated well among multiple assays for all three of the indicator species (Table 2.2). Based on these reproducible results, dosed lake water was considered a valid reference material for these studies. Due to depletion of the [^{14}C]-radiotracer in the first assay, a second dosed lake water set of exposures for *C. riparius* with endrin was not run (Table 1.4). Also, the dosed lake water exposures that used dual tracers for the DDT/chrysene study with *Diporeia* spp. showed a particularly high mortality (100% mortality by 96 hours), and this data set was not used to determine uptake rate coefficients.

Porewater and Sediment Control Data

Centrifuge tubes containing dosed whole sediment controls showed porewater contaminant concentrations to be comparable to test porewater contaminant concentrations in early exposure periods. However, porewater contaminant concentrations in test beakers tended to decrease from that in control tubes after 48 hours in most assays, most likely due to contaminant accumulation in the organisms.

Total organic carbon content in whole sediment exposures did not change significantly over the time intervals of any studies. Total organic carbon in the Lake Michigan sediment ranged from 0.45% to 0.52% in all assays. Organic carbon in some of the aqueous exposures decreased appreciably during some of the assays and adjustments for these decreases were made in accumulation calculations.

Total Lipid and Biotransformation Studies

Total lipid content of the three indicator species was determined to better describe possible differences in accumulation among the organisms studied and to determine if lipid content changed during the course of bioassays. For example, an appreciable amount of lipid lost during a bioassay might indicate a deterioration in general health of the species during the assays.

Baseline lipid content (percent dry animal weight) in *C. riparius* averaged 3.72 $\pm$ 2% S. D. in animals taken directly from culture aquaria. After exposure to the various assay media (sediment, dosed lake water, elutriate, or porewater) for 3 days, lipid content ranged from 0.37 to 9.90%, with a mean of 4.73%, not significantly different from baseline content. Baseline lipid content in *Diporeia* spp. taken directly from holding tanks averaged 33.91 $\pm$ 9.4% S. D. After 2 weeks exposure in

sediment or dosed lake water, no change in *Diporeia* spp. lipid was observed, where total lipid ranged from 23.7 to 44.49% and averaged 33.5% lipid.

Total lipid content in *L. variegatus* tended to decrease upon exposure to the media. Baseline lipids in this species taken from culture media averaged 10.09 ± 4.4% S. D. After three-to seven-day exposures in either porewater, dosed lake water, or whole sediment, lipid content declined to 2.46 to 9.11%, with an average of 5.43% lipid (A complete data set on lipid content of the three species is shown in Table B-I).

Biotransformation studies showed that of the three species tested with *trans*-chlordane, BaP, and pyrene, only *C. riparius* was able to biotransform BaP and pyrene to any extent (see complete data set for metabolite studies in Table C-I). After four-day exposures in whole sediment at 10°C, *C. riparius* larvae biotransformed 96.9% of the parent BaP and 71.5 to 73.6% of the parent pyrene to less hydrophobic metabolites, but transformed none of the *trans*-chlordane in dosed sediment. Neither *Diporeia* spp. nor *L. variegatus* were able to metabolize any of the three contaminants, and over 90% of the parent contaminants were detected in animal tissue after four- to 14-day exposures in dosed lake water or whole sediment.

Studies on aged sediment showed no significant transformation of the parent contaminants. After 6.5 months, the sediment still contained an average 92.8% parent BaP and 93.5% parent *trans*-chlordane, and after 2 months, single-labeled pyrene-dosed sediment contained an average 97.0% parent compound.

Accumulation from Aged Sediments

In assays that used *C. riparius*, contaminant accumulation appeared to either decline or stay the same with the amount of sediment/contaminant contact time, and was dependent upon the contaminant examined. Mean accumulation of *trans*-chlordane from whole sediment exposures after an eight-week settling period declined to about half that of sediment that had settled for one week even though sediment concentrations were similar (see accumulation data sets in Table A-I). Mean accumulation from whole sediment was comparable between eight-week and 26-week settling periods. Also, *trans*-chlordane accumulation from elutriate prepared from sediment aged from one and 26 weeks stayed relatively constant. However, porewater accumulation from sediment aged one week was much lower than that produced from sediment aged 26 weeks (Table A-I). Uptake rates of *trans*-chlordane from whole sediment aged one, eight, and 26 weeks appeared to decline in a linear fashion, while uptake from the aqueous extracts did not appear to change significantly with sediment aging (Table 2.5).

TABLE 2.5

K_{uoc} VALUES FOR *C. riparius* EXPOSED TO SEDIMENT AGED FROM ONE WEEK TO 6.5 MONTHS, AND AQUEOUS EXTRACTS GENERATED FROM AGED SEDIMENT

Compound	Media	Aging Interval		
		1 Week	8 Weeks	26 Weeks
trans-chlordane	whole sediment	358.0[a]	233.5	139.1
		(25)[b]	(16)	(13)
	elutriate	66.6	24.9	60.9
		(6)	(2)	(6)
	porewater	80.1	40.4	60.5
		(15)	(3)	(4)
BaP	whole sediment	15.73	23.49	12.57
		(1.4)	(3.0)	(1.4)
	elutriate	2.72	1.20	6.64
		(0.3)	(0.2)	(1.0)
	porewater	3.25	11.18	0.86
		(0.2)	(0.6)	(0.1)

[a]units for K_{uoc} are: $\frac{\mu g \text{ organic carbon cleared}}{\text{gram organism/hour}}$

[b]$\pm$ 1 standard error

Uptake rate coefficients of BaP did not reflect the accumulation decline from whole sediment exposures observed between one- and eight-week aging periods for *trans*-chlordane (Table A-III and Table 2.5). Mean accumulation of BaP in whole sediment declined by a factor of three in sediment aged one week versus that aged eight weeks. For example, mean *C. riparius* accumulation from whole sediment at 72 h was 1,030.3 $\pm$ 273 S. D. in sediment aged for one week. Accumulation from sediment stored for eight weeks prior to the study averaged 353.1 $\pm$ 123. However,

K_{uoc} was 15.73 ± 1.4 S. E. versus 23.49 ± 3.0 for whole sediment exposures that used sediment aged for one- and eight-week periods, respectively (Table 2.5). Also, accumulation of BaP from porewater that had been generated from sediment aged for 26 weeks was generally greater than porewater generated from sediment aged for one or eight weeks. However, uptake rate coefficients for porewater declined with sediment aging from 3.25 ± 0.2 S. E. (1 week) to 0.86 ± 0.1 (26 weeks; Table 2.5).

Uptake rate coefficients from elutriates generated from aged sediment followed trends in accumulation upon sediment aging, where both accumulation and K_{uoc} doubled with sediment aged one week and 26 weeks, respectively (Table 2.5 and Table A-III).

Uptake rate coefficients for *C. riparius* exposed to pyrene that used porewater generated from aged sediment increased with sediment aging. The K_{uoc}s were 85.9 ± 7 S. E. and 131.5 ± 20 for porewater exposures generated from sediment that had aged one and four weeks, respectively. No significant changes in pyrene uptake were observed for whole sediment and elutriate exposures. The K_{uoc}s for whole sediment were 165.9 ± 9 S. E. and 227.1 ± 50, and were 61.4 ± 4 and 55.7 ± 5 for elutriates produced from sediment aged one and four weeks, respectively.

Discussion

Exposure Comparisons

Data from this study show that aqueous extracts of whole sediment did not accurately represent the exposure observed in whole sediment. Generally, the aqueous extracts of whole sediment under-exposed organisms, compared to whole sediment, even after adjusting accumulation to the fraction of organic carbon contained in the test media. However, at some sampling times for some contaminants, differences in accumulation between a particular aqueous media and whole sediment were not significant, as was shown with nearly all pyrene-dosed elutriate exposures.

Results of the two-factor ANOVA and Student's *t*-tests used to analyze accumulation differences among the exposure media show that statistical significance of bioaccumulation data can be demonstrated in a number of ways. For ANOVA that used entire data sets for a single bioassay (*e.g.*, all accumulation values for whole sediment, elutriate, and porewater over all exposure intervals), accumulation was significantly different among exposure media for all but chrysene. Interaction effects between exposure media and sampling time were also seen in the ANOVA. However, when single timed exposures were anlayzed for accumulation differences between whole sediment/elutriate and whole sediment/porewater

exposures, significant differences were not apparent for some sampling intervals. When determining which statistical method is the most suitable for analyzing bioaccumulation data such as these, one must keep in mind the biological as well as the statistical significance of the results. In typical bioassays where only a single exposure interval is examined, a wide variation among individual animals may create no statistical significance between test and control exposures, while significant differences may be observed over a temporal interval of the dose-response.

Sampling time was a significant factor for bioaccumulation relationships among whole sediment and aqueous media, exemplified by DDT accumulation in *Diporeia* spp. DDT accumulation from porewater and elutriate exposures both correlated with accumulation from whole sediment early in the assays. However, after 72-h exposure intervals, both aqueous exposures underestimated DDT accumulation from whole sediment. Sampling time should be considered when determining the suitablility of aqueous extracts of sediments as substitutes for whole sediment exposures, such as in toxicity identification evaluations. In aqueous exposures, a steady-state contaminant concentration between the organism and its environment may never be attained, since lack of substrate or limited nutrients may dictate the length of time that the exposure interval can be maintained before organism die-off. Therefore, an important factor to consider in methods development is the length of exposure interval that will produce the most reliable results, which will most likely vary with the indicator species used. In preliminary studies, detrimental changes (*i.e.*, loss of color and decreased movement) were observed in *C. riparius* after 96-h exposures in aqueous media. However, *Diporeia* spp. could be exposed for over two weeks in porewater or elutriate without obvious effects. Thus, exposure times should be determined in accordance with individual species tolerances to exposure conditions. In most assays, elutriate:sediment and porewater:sediment accumulation ratios followed obvious trends over sampling intervals. Accumulation ratios usually decreased with the exposure time. However, trends increased with time in some assays (*e.g.*, *C. riparius* exposed to endrin, *L. variegatus* exposed to pyrene). One explanation for the contaminant in porewater and/or elutriate becoming less available relative to whole sediment may be that the available fraction of the contaminant became depleted in aqueous media after a short time while source contaminant in sediment exposures remained relatively constant. While the total contaminant concentrations in the sediment stayed constant, total concentrations decreased appreciably in porewater and elutriate over

the course of the assays, and it is likely that the bioavailable concentrations in these media also decreased.

This fails to explain the upward trend in temporal accumulation ratios in other assays. A number of factors may have contributed to this apparent increase in aqueous bioavailability relative to contaminant availability in whole sediment. The accumulation data were normalized to the total amount of organic carbon (both dissolved organic carbon and particulate organic carbon) contained in bioassay media, since the activity of hydrophobic contaminants is controlled primarily by their interaction with organic carbon (McCarthy 1983, DiToro *et al.* 1991). By normalizing to organic carbon, I directly accounted for the carbon-bound fraction of contaminant, and thus indirectly accounted for the freely dissolved concentrations in aqueous phases (*e.g.*, isolated porewater or elutriates), or porewater in whole sediment. This was done because: (1) an adequate approximation of the amount of bioavailable (*e.g.*, freely dissolved fraction) contaminant could not be made from whole sediment exposures, and (2) in preliminary studies with aqueous media, bioaccumulation appeared to be much greater than that contributed by the freely dissolved fractions alone. Although such normalizing techniques may work well for some contaminants of low solubility that are tightly bound to carbon, it may not be as useful for other contaminants that are more water-soluble and less tightly bound to organic carbon. Pyrene and endrin were the most water-soluble contaminants used (pyrene = 135.0 µg/L, Mackay *et al.* 1980; endrin = 230.0 µg/L, Hayes and Laws 1991), and were the only contaminants that demonstrated a significant upward trend in accumulation ratios over time for *L. varigatus* (pyrene exposures) and *C. riparius* (endrin exposure). This upward trend demonstrates increased exposure of the contaminants with time, as may happen when less hydrophobic compounds slowly desorb from organic carbon to the surrounding aqueous medium.

Differential exposure among whole sediment, elutriate, and porewater was clearly demonstrated in the uptake clearances, where, for most contaminants, K_{uoc}s in aqueous extracts were lower than those obtained from whole sediment exposures by at least a factor of two. The only exceptions were with *Diporeia* spp. exposed to DDT and chrysene in a dual-labeled assay. In that assay, K_{uoc}s in porewater exposures were approximately two times greater than those in whole sediment. Reasons for this phenomenon are unknown, and further assays that test these contaminants separately may indicate why the uptake from porewater was greater than uptake from whole sediment.

Species Effects

In this study, sediment possessing the same chemical and physical characteristics and containing the same organic carbon content was used. All studies were conducted at 10°C so that an adequate comparison of contaminant uptake could be made among all three of the indicator species. However, variations in uptake and accumulation among the species were still apparent. Where direct comparisons in accumulation among all three species could be made (pyrene, BaP, and *trans*-chlordane), elutriate:sediment and porewater:sediment accumulation ratios generally varied both in magnitude for a single sampling time as well as temporally. In most cases, *C. riparius* had lower accumulation ratios than either of the other two species, while *Diporeia* spp. generally showed the greatest accumulation for a given length of exposure. However, contaminant clearances among the species were generally comparable among species for a single contaminant in sediment, elutriate, or porewater.

Increases or decreases in contaminant accumulation among the three species probably resulted from a combination of feeding selectivity, lipid content, and physiological/metabolic variation. Contaminant uptake from both gill ventilation and ingestion of food is thought to contribute to contaminant body burden (Adams 1987, Landrum and Stubblefield 1991, Lee 1992). A greater contaminant accumulation contributed from food ingestion may occur with selective feeding habits, as seen with *Diporeia* spp. If contaminant differentially sorbs onto particles that *Diporeia* preferentially ingests, exposure is increased and accumulation increases accordingly. Also, the higher lipid stores in *Diporeia* may increase its ability to accumulate lipophilic compounds. *Diporeia* spp. contained an average ten times more lipid on a dry weight basis, compared to *C. riparius*. Since hydrophobic contaminants partition preferentially to lipid, the potential for *Diporeia* spp. to accumulate these compounds was expected to be much greater than for either of the other two species assayed. In some cases, this was true (*e.g.*, pyrene accumulation from sediment, elutriate, and porewater). However, in other cases, accumulation was greatest in *L. variegatus* (*e.g.*, accumulation of *trans*-chlordane in sediment), where this species' continuous feeding habits probably contributed to the significantly higher accumulation of BaP from whole sediment compared to that observed for the other two species.

Differences in metabolism among the indicator species may have also been responsible for the differential accumulation observed for a single contaminant. Metabolic studies were conducted on the three species primarily to determine if re-

uptake of metabolite(s) from the exposure media was a significant source of contaminant in the organisms. While re-uptake was not considered to be a significant contributor to overall accumulation, only a portion of the accumulated pyrene and BaP in *C. riparius* was parent compound. I assume that the rate of biotransformation in *C. riparius* remained the same in all exposure media. The accumulation differences observed in *C. riparius* exposed to BaP and pyrene in porewater, elutriate, and whole sediment are valid only if this assumption is true. However, the pyrene and BaP elutriate:sediment and porewater:sediment accumulation ratios obtained for *C. riparius* tend to agree with those I obtained for non-metabolized *trans*-chlordane; all of these ratios were generally lower than those obtained for *Diporeia* spp. or *L. variegatus*. Therefore, PAH metabolites in *C. riparius* apparently did not alter bioaccumulation among the exposure media, and the clearance and accumulation comparisons that I make among the exposure media are valid.

Physiological differences among the species, including the ability to survive in the various exposure media for extended periods, may have contributed to the differential bioaccumulation of single contaminants. Abnormal stresses were placed on the organisms during exposures in aqueous media. All three species normally inhabit sediments, so lack of a suitable substrate was expected to change behavior and possibly the rate of contaminant uptake. Previous studies showed toxicity of an oil leachate to burrowing mayfly larvae decreased when artificial burrows were supplied in exposure chambers (Henry *et al.* 1986). The authors suggested that the increased toxicity seen in chambers without burrows was due to thigmotactic stress created from lack of a suitable substrate. *Chironomus* spp. normally build larval cases on or within the substrate in which they live (Oliver 1971). However, no cases were constructed in aqueous exposures because no suitable material was available. This may have acted to either raise or lower the contaminant exposure from the surrounding water to the larvae. Cases tend to "wall" the organism off from the surrounding sediment in natural situations, and are expected to reduce the organism's direct exposure to porewater (Lee 1991). In aqueous exposures, the organisms were no longer sheltered from contaminant contact provided by the case, and exposure was raised. However, exposure may have also been lower than when cases were present if the animals lowered their feeding rates in aqueous media. The stress brought about by an unsuitable substrate likely affected *L. variegatus*, where mean lipid content dropped to half that of organisms taken from culture media after three- to seven-day exposures.

A relatively wide variation of contaminant uptake within individual animals at individual sampling times occurred in most of the assays. Individual variation in contaminant uptake is indicative of differential exposure of organisms to the contaminants, due to normal biological variation in feeding rates, respiration, age, and general metabolic rates among organisms. Even though test organisms were carefully selected and sized before being placed into exposures, it was not possible to determine the exact age or sex of individuals. Fourth instar *Chironomus* larvae may be at very different stages of development (Wülker and Götz 1968), and may likely slow their feeding late in this period. *Diporeia* spp. are intermittent feeders and normally show individual variation in feeding rate (Quigley 1988, Harkey *et al.*, 1993*a*). Finally, previous accumulation assays showed that contaminant uptake naturally varies among individual *L. variegatus*, and that for whole-sediment accumulation tests, both longer exposures and more sample replicates should be used (Phipps *et al.* 1993).

Compound Effects

The relative magnitude of the uptake rate differences observed among contaminants such as *trans*-chlordane and BaP are attributable to differential bioavailability inherent to the compounds studied. Uptake rate coefficients in whole sediment exposures were highest for *trans*-chlordane, ranging from 216.8 (*Diporeia* spp.) to 358.0 (*C. riparius*), and were lowest for BaP, ranging from 11.0 (*Diporeia* spp). to 53.5 (*L. variegatus*). Differential bioavailability may arise from the contaminant partitioning to different particle-sizes within the sediment or to different types of organic carbon (*i.e.*, colloids, micro-particulates). This was apparent in studies with *Diporeia* spp. where differential association of hexachlorobiphenyl and BaP to TOC within the same sediment led to differential contaminant exposure (Harkey *et al.*, 1993*a*).

The high uptake observed in endrin exposures conducted at effects-level doses was probably due to stimulatory reactions of the *C. riparius*, inherent to the contaminant (Klaassen 1985). I diluted the high-dose sediment to an assumed no-effect level of contaminant (*e.g.*, no mortality, behavioral changes), and K_{uoc} decreased from 1,726.9 to 189.2 in whole sediment exposures. Even though mortality was not observed at the lower endrin concentration, pyrene uptake in this dual-labeled assay was over four times greater than when *C. riparius* were exposed to pyrene in single-labeled assays, indicating that even at seemingly no-response levels, organism metabolic rate was affected, probably by the endrin. Other studies showed

that the toxicity of endrin to *Hyalella azteca* was not affected by the amount of TOC in the exposure, and that, for this contaminant, normalization to organic carbon is of limited use (Nebeker *et al.* 1989).

I found the bioavailability of contaminants from whole sediment, porewater, and elutriate to decrease, increase, or stay the same with the length of sediment/contaminant contact time. Bioavailability, as measured by K_{uoc}, appeared to be both a function of the exposure media as well as the contaminant studied. Uptake of *trans*-chlordane by *C. riparius* in all three media appeared to decline with the age of sediment contamination. These data agree with previous partitioning studies that showed the freely dissolved fraction of *trans*-chlordane to decline in elutriate and porewater generated from sediments aged from seven to 60 days (Harkey *et al.* 1993*b*). However, those partitioning studies, as well as other studies that measured uptake clearances did not establish clear linear relationships between sediment aging and the amount of biologically-available BaP and pyrene (Landrum *et al.* 1992*c*). The mechanism for these changes in contaminant bioavailability are not known, and further studies engaging laboratory-dosed and field-collected sediments are needed before cause and effect relationships can be established. However, the effect of aging on bioavailability is an important variable and should be considered when determining hazard represented by laboratory-dosed sediments as well as in-place pollutants.

These data demonstrate the difficulties encountered when comparing the bioavailability of a number of hydrophobic organic contaminants in whole sediment and aqueous fractions of whole sediment, and the assumptions that must be made pertaining to partitioning of contaminants to organic carbon. While normalizing the data to the fraction of TOC in the exposure media is considered to "even out" differences in bioavailability (Ziegenfuss *et al.* 1986, U. S. EPA 1989*a*), the data suggest that normalization to TOC may be more appropriate for some contaminants than for others. In addition, the differential bioavailability of contaminants arising from sediment/contaminant contact time or sediment dilution may affect organic carbon/contaminant partitioning, and weakens the theory that increased levels of TOC will mediate the bioavailability of organic contaminants.

The data also cast doubt on using benthic invertebrates in aqueous media to simulate whole-sediment exposure. Whereas these species may be adequate indicators of hazard in substrate similar to their natural benthic environment, other species native to water-column or epibenthic environments may be more suitable for aqueous studies. Water-column species, such as *Daphnia* or *Ceriodaphnia* spp. may

not be appropriate for assessing the toxicity of sediment-associated contaminates. Certainly, trade-offs between what species will "work" and what species is most indicative of hazard represented by sediment-associated contaminants will have to be made if future bioassay techniques that use aqueous fractions of whole sediment are to be developed.

PARTITION COEFFICIENTS OF HYDROPHOBIC CONTAMINANTS IN NATURAL WATER, POREWATER, AND ELUTRIATES OBTAINED FROM DOSED SEDIMENT: A COMPARISON OF METHODOLOGIES

Introduction

Toxicokinetic models have been used to predict contaminant exposure to organisms for both aqueous and sediment bioassays (Landrum *et al.* 1992 *a*). One such frequently used first-order rate coefficient model determines the contaminant flux into an animal as dependent on the contaminant concentration in a source compartment, typically expressed as (uptake clearance x source compartment concentration) - (animal elimination x animal concentration) (Rand and Petrocelli 1985). For sediment exposures, porewater has been suggested as the source compartment (Adams 1987, Kemp and Swartz 1988, U. S. EPA 1989*a*). Following this assumption, numerous bioassays have utilized aqueous test fractions, either of porewater obtained from whole sediment or of elutriates (extracts of whole sediment originally intended to mimic suspension events associated with dredging operations; see Giesy and Hoke 1989 for a review). To account for the actual exposures in assays that use these aqueous test fractions, an accurate assessment of the bioavailable form of contaminant is necessary. Current theory suggests that the freely dissolved contaminant is the only form available to an organism residing in the aquatic environment for accumulation (Landrum *et al.* 1985, Kukkonen *et al.* 1990). The remainder that is bound to organic material, colloids, and other microparticulate material is unavailable over relatively short exposures (*e.g.*, hours; Voice *et al.* 1983, Chin and Gschwend 1992). If the organism is a filter feeder, some of the material on small particles or colloids may well be available through ingestion. Bound and freely dissolved phases have been measured through a variety of methods, including equilibrium dialysis (Carter and Suffet 1982, Kukkonen *et al.* 1990), C-18 reverse phase separation (Landrum *et al.* 1984, Maaret *et al.* 1992), water solubility enhancement (Chiou *et al.* 1986), and flourescence techniques (only suitable for fluorescent compounds; Roemelt and Setiz 1982, Schlautman and Morgan 1993). Although these methods may work well for organic contaminants at relatively high concentrations, they may not be as efficient when applied to hydrophobic organics in nanomolar quantities.

Partitioning can be quite variable in both porewater and natural waters for a single contaminant, even when corrected for the amount of organic carbon in the

system (Landrum *et al.* 1985, 1987). Variation in the composition and quantity of the organic material present and the extent of laboratory manipulation involved in obtaining porewater or in dosing natural water may be responsible for the observed variability in partitioning. Further, the relative amounts and/or composition of bound and freely dissolved fractions may change during the course of a bioassay, due to the behavior of the bioassay organisms or as a result of bioassay conditions (*i.e.*, feeding). If changes in contaminant bioavailability occur and are not considered in the bioassay or toxicokinetic model, the rate of contaminant flux and thus the potential for bioaccumulation and subsequent effects may be inappropriately estimated or misunderstood.

This study examined the partitioning of organic contaminants in elutriates and porewater obtained from laboratory-dosed whole sediment and laboratory-dosed lake water. I compared partition coefficients generated from four methods to determine which methods were most suitable for estimating the bioavailable, "freely dissolved" fraction of contaminant. In addition, I wanted to determine any differences in partitioning resulting from sediment aging and manipulation, as well as any differences that may occur over various exposure intervals in a typical bioaccumulation assay.

Materials and Methods

Chemicals

The compounds studied included ^{14}C-radiolabeled *trans*-chlordane (13.7 mCi/mmol, Velsicol Chemical Co., Memphis, TN), [^{14}C]-endrin (8.4 mCi/mmol, Sigma Chemical Company, St. Louis, MO), [^{3}H]-benzo(*a*)pyrene (BaP, 40.0 Ci/mmol, Sigma Chemical Co.; 69.0 Ci/mmol, Amersham Ltd., Amersham, UK), and [^{3}H]-pyrene (25.2 Ci/mmol, Chemsyn Science Laboratories, Lenexa, KS). All compounds were dissolved in an acetone carrier. Compound radiopurity was greater than 97% for all compounds prior to use, as determined by thin layer chromatography, using either benzene:ethyl acetate (3:1, v:v, endrin and *trans*-chlordane) or hexane:benzene (8:2, v:v, BaP and pyrene) and liquid scintillation counting (LSC). Analytical procedures were performed under gold fluorescent light ($\lambda \geq 500$ nm) to minimize the photodegradation of the polycyclic aromatic hydrocarbons (PAHs).

Sediment Dosing and Manipulation

Sediment was collected at a 45-m depth in Lake Michigan by Ponar grab approximately 8 km off the coast of Grand Haven, MI. The sediment was passed through a 1-mm sieve to remove debris and indigenous organisms. A sediment-water

slurry was made by diluting wet sediment with Lake Michigan water in a 1:4 sediment to water ratio (w/v). Radiolabeled chemicals were added to the slurry drop by drop in a minimal amount of acetone carrier (<1 ml per liter wet sediment) while being stirred on a mechanical stirrer at room temperature for 4 h. Three sediments were prepared in the following combinations and range concentrations: BaP/*trans*-chlordane (BaP = 0.39 ng/g dry weight, *trans*-chlordane = 1.66 μg/g), pyrene/endrin (pyrene = 0.87 ng/g, endrin = 0.53 μg/g), and pyrene/*trans*-chlordane (pyrene = 0.64 ng/g, *trans*-chlordane = 1.74 μg/g), where one compound was ^{3}H labeled and the other was ^{14}C labeled. One sediment was dosed only with pyrene (0.76 ng/g dry weight). After stirring, sediment slurries were allowed to settle at 4°C for 48 h. Subsequently the overlying water was decanted, and the sediment was washed with another four volumes of lake water by stirring for another 4 h and settling at 4°C for 48 to 96 h. The overlying water was again decanted, and portions of the sediment were either used to prepare elutriates or centrifuged to obtain porewater for the various partitioning procedures.

Elutriate Preparation

A 1:4 ratio (sediment:water, v:v) of prepared dosed sediment was used to prepare elutriates (U. S. EPA/CE 1990). The sediment/water mixtures were placed into 250-ml Teflon centrifuge bottles. The bottles were placed on a laboratory rotator at 200 rpm for 30 min, then were allowed to settle for 1 h. The unsettled portions were decanted into clean Teflon centrifuge bottles and were centrifuged at 2000*g* at 10°C for 30 min. The elutriate fractions were decanted and stored for no longer than 24 h at 10°C before use in partitioning studies. Contaminant concentrations in elutriates ranged from 0.24 pg/ml (pyrene) to 54.8 ng/ml (*trans*-chlordane).

Porewater Preparation

Dosed prepared sediment was placed into 250-ml stainless steel centrifuge bottles and centrifuged at 4000*g* for 30 min at 10°C. Supernatant from the bottles was decanted into 250-ml Teflon centrifuge bottles and was spun at 2000*g* for 30 min at 10°C. The supernatant from all bottles was decanted and stored for no longer than 24 h at 10°C before use in partitioning studies. Contaminant concentrations in porewater ranged from 0.67 pg/ml (BaP) to 16.24 ng/ml (*trans*-chlordane).

Dosed Lake Water Preparation

Lake Michigan surface water was collected about 1 m below the surface and stored at 4°C until used. Quantities of lake water were filtered through glass fiber filters (Gelman type A/E, Gelman Sciences, Ann Arbor, MI), then through 0.45-mm

polycarbonate filters (Nuclepore Corp., Pleasanton, CA) to remove particles. Containers of lake water were placed on a laboratory stirrer after the addition of radiolabeled compounds in acetone carrier. Compounds were added with the same compound combinations for the dosed sediments in concentrations ranging from 0.15 pg/ml (pyrene) to 37.12 ng/ml (*trans*-chlordane). Mixtures were stirred at room temperature for at least 1 h and were stored overnight in the dark at 10°C before use in partitioning studies.

Organic Carbon Measurements

Organic carbon in lake water, porewater, and elutriate exists in particulate, colloidal, and dissolved forms. Both filtration and centrifugation can remove some of this, depending on the filter size and centrifugal force employed. However, I chose to define all organic carbon in whole porewater, elutriate, and lake water samples as TOC, since a definite measure of organic carbon associated with colloids and microparticulates that may have remained after isolation and pre-treatment processing (*i.e.*, filtration and centrifugation) could not be determined. Aqueous measures of total organic carbon (TOC) from elutriate, porewater, and dosed lakewater were performed on an Oceanography International® carbon analyzer after persulfate digestion (Golterman *et al.* 1978). Duplicate analyses yielded differences of 1 - 10%. Replicate samples of prepared lake water, elutriate, or porewater were taken prior to each method evaluation for determination of TOC in the total aqueous material.

Sampling and Chemical Analyses

Aqueous samples for compound concentrations were removed in 2-ml aliquots and placed directly into 12 ml scintillation cocktail (Research Products International 3a70b, Mt. Prospect, IL). Radioactivity was determined via LSC on an LKB 1217 liquid scintillation counter. The data were corrected for quench using the external standards ratio method after correcting for background.

Method Evaluation

Four methods were used to determine partition coefficients. The following compounds were used to compare filtration and centrifugation methods: [^{3}H]-BaP, [^{3}H]-pyrene, [^{14}C]-*trans*-chlordane, and [^{14}C]-endrin. In addition, lake water and elutriate containing dual labeled [^{14}C]-*trans*-chlordane and [^{3}H]-BaP were used to evaluate the XAD resin methods, while elutriate from [^{3}H]-BaP dosed sediment was used in the dialysis method.

XAD Resin Method

The polymeric resin XAD-4 was used to evaluate the separation and recovery of the compounds because it incorporates the properties of high surface area, porosity, and hydrophobicity (Landrum and Giesy 1981). Two methods used XAD-4. In one method, champagne columns 1.5 cm diameter x 25 cm long were plugged with glass wool and packed with 25 $\pm$ 2 ml of wet precleaned Amberlite XAD-4 resin (Rohm and Haas, Philadelphia, PA). After pre-conditioning the columns with 5 ml deionized water, 10-ml replicate samples of elutriate or dosed lakewater were percolated through the columns. After percolation, the columns were rinsed with 5 ml deionized water. The filtrate was collected and a sample was taken for LSC. This fraction was defined as the bound fraction. The columns were sequentially eluted with 10 ml acetone. Samples of the eluted fraction, defined as the freely dissolved fraction, were taken for LSC. The percent of bound compounds was calculated as the activity of compounds from the column filtrate divided by the activity of compound present in the sample prior to percolation through the column. Mass balance was calculated as the total amount of compound percolating through and eluted from the columns divided by the amount of compound present in the sample prior to percolation through the columns.

In an alternate, XAD-4 "stirred" method, 0.3 g precleaned XAD-4 resin was mixed with 10 ml of elutriate in 20-ml glass scintillation vials. The vials were manually shaken for 60 s, then left to settle. Two ml of supernatant was removed and activity determined via LSC. The remaining resin was removed by vacuum filtration through glass fiber filters (type GF/C, Whatman Ltd., Maidstone, England). The filter and resin were dried in a 90°C oven for 20 min, then placed into scintillation cocktail for analysis via LSC. Freely dissolved and bound fractions were determined from resin/filter and supernatant respectively, as described for the "column" method.

Equilibrium Dialysis

The procedure for dialysis followed the methods of Carter and Suffet (1982). Distilled water was dosed with radiolabeled contaminant and mixed for 2 h in the dark at room temperature. Contaminants were directly spiked into distilled water to hasten equilibration of contaminant between elutriate (inside membrane) and the surrounding water outside the dialysis membrane. Two hundred ml of dosed water was dispensed into 250-ml brown bottles. Sodium azide (0.002%) was added to inhibit microbial growth. Nine cm lengths of 10-mm diameter dialysis tubing (Spectra/Por®

cellulose ester membranes, Spectrum Medical Industries, Los Angeles, CA) were soaked in deionized water to remove the sodium azide preservative. The lengths were filled with 4 ml prepared elutriate and secured with clamps. Each dialysis bag was placed into one of the prepared brown bottles and was sealed with a Teflon-lined cap. Bottles were placed on a laboratory rotator at 100 rpm at room temperature. Controls consisted of dialysis bags filled with 4 ml distilled water instead of the elutriate. Three replicate bottles and one control were removed from the rotator at 48, 99, and 170 h. Replicate 2 ml samples were collected from both dialysis bags and the bulk water and analyzed via LSC.

Freely dissolved contaminant was calculated from the bulk water activity (outside the membrane), while the difference between activities inside and outside the membrane was defined as the bound fraction. Mass balance was determined as the amount of chemical activity from the sum of concentrations inside, outside, and from a hexane rinse of the bottles and membranes that corrected for sorption to surfaces divided by the total activity of dosed water originally added to the bottles.

Filtration Method

The C-18 reverse-phase/filtration method followed a modified procedure described by Eadie *et al.* (1990). Replicate samples of prepared lake water, elutriate, or porewater were analyzed for total contaminant activity via LSC. Fifteen-ml aliquots of the aqueous solutions were then filtered through two 25-mm glass fiber filters, using a stainless steel filter support. The top filter collected particulate and dissolved matter, while the bottom filter was presumed to adsorb an equal amount of dissolved organic material. Each filter was placed into a scintillation vial with 12 ml scintillation cocktail and sonicated for 30 s, using a high intensity probe sonicator. Filters were counted to correct for the particulate and dissolved fractions of contaminant(s). The filtrate was sampled for contaminant activity (defined as the particulate-free fraction), and 10 ml was passed through a Waters® C-18 reverse phase Sep-Pak column (Millipore Corp., Milford, MA) that had been pre-rinsed with 5 ml of filtrate. Samples of this filtrate, defined as the bound fraction, were analyzed for contaminant activity via LSC.

The bound contaminant fraction was calculated as

$$\frac{\text{top filter activity - (2 x bottom filter activity)}}{\text{\# ml filtered}} + \text{activity of C-18 filtrate,} \quad (1)$$

where activity = dpm/ml. The freely dissolved fraction was calculated as

filtrate activity after glass fiber filters - filtrate activity after C-18 column

$$+ \frac{(2 \times \text{bottom filter activity})}{\#\ \text{ml filtered}}. \quad (2)$$

Percent of bound and freely dissolved contaminants was calculated as the activities of either freely dissolved or bound phases divided by the total activity in the sample determined prior to filtration.

Centrifugation Method

Samples of prepared lakewater, elutriate, or porewater were analyzed for compound activity via LSC. Forty ml of the aqueous phases were placed in 50-ml stainless steel centrifuge tubes and were centrifuged at 20,000*g* for 30 min at 10°C. Supernatant was sampled for TOC and 10 ml were passed through a C-18 Sep Pak column. The bound fraction of contaminant in the centrifuged sample was defined as the activity of contaminant in the fraction that passed through the column. The freely dissolved fraction was calculated by subtracting the bound fraction activity from the total activity found in the supernatant. Mass balance calculations were made by dividing the activity of supernatant fractions, centrifuged pellet, and acetone rinse of the centrifuge tubes by the activity of the contaminant in the samples prior to centrifugation.

Calculation of Partition Coefficients

Partition coefficients, defined as K_p values, were corrected for aqueous TOC. All aqueous phases were sampled for TOC determination before partitioning manipulation with the exception of the centrifugation method, where TOC was sampled from the supernatant after centrifugation at 20,000 *g*. Partition coefficients were calculated as

$$\frac{\text{activity of bound contaminant (ng/ml) / TOC (mg carbon/ml)}}{\text{activity of freely dissolved contaminant (ng/ml)}}. \quad (3)$$

Sediment Manipulation and Aging

Partitioning of contaminants in porewater and elutriates generated from four sediments dosed with BaP/*trans*-chlordane, pyrene/endrin, and pyrene (2 separate batches) was examined using the centrifugation method. The sediment had previously been dosed and used in bioassays that utilized whole sediment exposures. After the exposures, bioassay organisms were removed from the sediment. Sediment from all exposure containers was combined, mixed, and placed in the dark at 4°C for various

aging periods before partitioning was determined. After partitioning studies were performed on porewater and elutriates produced from one of the aged sediments (pyrene/endrin), the sediment was again recovered and stored in the dark at 4°C. One week later, porewater and elutriates were again generated from the sediment, and partitioning of the contaminants was examined.

Bioassay Procedures

Samples of dosed lake water, elutriate, and porewater were prepared and used in bioaccumulation assays. Two fourth instar *Chironomus riparius* larvae were subjected to 20 ml of the aqueous media in static exposures at 10°C. At the completion of predetermined exposure intervals (one-, six-, 24-, 48-, and 72-h), samples were analyzed for TOC and amounts of bound and freely dissolved contaminants using the filtration method described above. Partition coefficients were calculated and used to evaluate any changes in partitioning over the course of the assay.

Statistics

Two-way analysis of variance was used to test for overall significant differences ($p < 0.05$) in partitioning among aqueous phases and methodologies (SAS® 1988). Scheffe's multiple range test was used for post-hoc multiple comparisons within categories. Differences between two means were compared by using Student's *t* tests. Differences were considered statistically significant when $p < 0.05$. Linear regression and correlation coefficients were used to evaluate differences in partitioning after sediment manipulation (Data Desk® 1985).

Results

Methods Evaluation

Equilibrium Dialysis

Recovery of the compounds tested using dialysis was low, where only 11 - 14% of total BaP activity could be found in bound and freely dissolved fractions after 170 h. Mean BaP concentrations inside and outside dialysis membranes were 0.34 ± 0.02 pg/ml and 0.13 ± 0.01 pg/ml, respectively, after 170 h. The percent of bound BaP in elutriates was much lower when measured by dialysis compared to the XAD and centrifugation methods, but was comparable to the filtration method (Table 3.1).

Dialysis was also attempted with elutriates in water dosed with *trans*-chlordane. However, even with intially high activities present in the water, activities from inside the dialysis bags after 170 h were close to background, and partition

TABLE 3.1

COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR BENZO(*a*)PYRENE, USING A VARIETY OF RECOVERY METHODS

Method	Dosed Lake Water			Elutriate			Porewater		
	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
XAD Column	4.66 (1.05)	45.6 (20.1)* n = 7	4.83 to 5.64	5.09	80.0 (11.2) n = 6	5.50 to 7.56	--**	--	--
XAD Stirred	--	--	--	5.09	90.4 n = 2	6.18 to 6.38	--	--	--
Dialysis	--	--	--	11.33 (3.64)	64.4 (6.5) n = 5	5.05 to 5.38	--	--	--
Centrifug. @ 20,000 *g*/Sep Pak	8.82 (4.05)	33.81 (6.8) n = 6	4.37 to 4.73	13.9	95.47 (3.3) n = 3	6.02 to 6.99	34.5	98.9 n = 2	6.40 to 6.41
Filtration/ Sep Pak	5.93 (1.02)	29.94 (10.8) n = 5	3.75 to 5.48	5.09	62.43 (23.7) n = 12	4.72 to 6.73	12.45	79.77 (11.0) n = 9	5.18 to 6.07

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment.

Analysis of dosed lakewater was performed within 24 hours of preparation.

*$\pm$ 1 S. D.

**Test not performed.

coefficients could not be evaluated. The loss of activity over the time course of the study was accounted for by sorption of compounds to glass walls of the bottles, plastic surfaces of the dialysis bag clamps, and the membranes themselves.

XAD-4 Resin

A wide range of total contaminant recovery was seen with the column method (54.3 - 103.2% for BaP; 45.9 - 90.4% for *trans*-chlordane), while recovery was greater for the stirred method (97.6 - 100.0% for BaP; 85.6 - 101.2% for *trans*-chlordane). However, the transfer, filtration, and drying of the resin in the stirred method proved to be much more time consuming than column method procedures. Partition coefficients produced from the column method were more variable in BaP-contaminated elutriates than for dialysis, centrifugation, or filtration methods (Table 3.1).

Filtration

The percent of BaP binding from the filtration method was lower than that obtained via XAD or centrifugation (Table 3.1). Percent of *trans*-chlordane binding was not significantly different between XAD and filtration methods (Table 3.2). Porewater bound the greatest percentage of BaP and *trans*-chlordane while lake water bound the least, even after adjusting for TOC, as reflected in K_p values (Tables 3.1 and 3.2). Conversely, most binding of endrin occurred in lakewater (69.51%), while elutriate and porewater bound considerably less (Table 3.3). A wide variation in the percent bound fraction of lake water with the filtration method occurred with pyrene (62.4 $\pm$ 31%; Table 3.3).

Centrifugation

Mean recoveries for the centrifugation method were high and ranged from 82.8% (pyrene) to 100+% (*trans*-chlordane). Sorption of compounds to the stainless steel centrifuge tubes was lowest for endrin (7.33 $\pm$ 2.7%), and was only slightly higher for the other three compounds examined: BaP 15.11%, *trans*-chlordane 17.76%, and pyrene 18.02%. The centrifugation method showed the highest mean percentage of bound BaP in elutriate and porewater (Table 3.1) and the least *trans*-chlordane and pyrene binding in lakewater, among the various methods examined (Tables 3.2 - 3.3). For endrin, the percentage of bound contaminant with centrifugation was only about one third that of the filtration method in elutriate, and about half that of filtration in porewater (Table 3.3). For the most part, the range of partition coefficients obtained with the centrifugation method compared with those obtained with other methods. However, log K_p values were approximately 70

TABLE 3.2

COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR *trans*-CHLORDANE, USING A VARIETY OF RECOVERY METHODS

Method	Dosed Lake Water			Elutriate			Porewater		
	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
XAD Column	4.66 (1.05)	47.8 (17.1)* n = 7	4.64 to 5.70	5.09	72.6 (11.0) n = 6	5.541 to 6.03	--**	--	--
XAD Stirred	--	--	--	5.09	60.4 n = 2	5.21 to 5.78	--	--	--
Centrifugation @ 20,000 *g*/Sep Pak	8.82 (4.05)	13.42 (3.1) n = 6	4.90 to 5.08	13.9	64.52 (3.4) n = 3	5.08 to 5.20	34.5	83.96 n = 2	6.89 to 6.98
Filtration/Sep Pak	6.42 (1.5)	40.34 (10.7) n = 5	4.82 to 5.38	5.09	60.20 (18.0) n = 13	4.88 to 6.18	13.34	73.28 (10.7) n = 7	4.97 to 5.80

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment. Analysis of dosed lakewater was performed within 24 hours of preparation.

* $\pm$ 1 S. D.

**Test not performed.

times higher for the centrifugation method, compared with filtration for *trans*-chlordane in porewater (Table 3.2).

TABLE 3.3

COMPARISON OF MEAN PERCENT BOUND AND PARTITION COEFFICIENTS (K_p) FOR ENDRIN AND PYRENE, USING SEP PAK SEPARATION FOLLOWING CENTRIFUGATION AND FILTRATION METHODS

Method	Dosed Lake Water			Elutriate			Porewater		
	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p	TOC mg/L	% Bound	log K_p
Endrin, Filtration	5.65 (2.5)*	69.51 (13.1) n = 11	4.54 to 5.79	11.34 (3.3)	37.93 (11.4) n = 9	3.25 to 4.91	21.8 (2.8)	54.65 (13.4) n = 10	4.41 to 5.14
Endrin, Centrifugation	--**	--	--	3.61 (0.3)	10.63 (0.7) n = 5	4.46 to 4.55	11.35 (0.9)	29.30 (6.7) n = 6	4.44 to 4.70
Pyrene, Filtration	7.08 (3.0)	62.43 (30.8) n = 15	3.96 to 6.80	11.34 (3.3)	58.85 (16.6) n = 10	4.79 to 5.68	21.8 (2.8)	65.14 (16.1) n = 11	4.55 to 5.64
Pyrene, Centrifugation	6.33	5.40 (0.8) n = 6	3.84 to 4.04	8.84 (9.1)	37.88 (6.4) n = 7	4.65 to 5.18	12.7 (2.4)	85.44 (4.9) n = 9	5.54 to 5.92

Partitioning analysis was completed within 24 hours of elutriate and porewater preparation from dosed sediment. Analysis of dosed lakewater was performed within 24 hours of preparation.

* ± 1 S. D.

**Test not performed.

Overall Methods Evaluation

No significant differences in the percent of bound BaP were determined among XAD, filtration, and centrifugation methods using two-way analysis of variance and post-hoc testing, although differences were seen among methods with the three other contaminants (Table 3.4). Significant differences in binding were seen among all aqueous phases contaminated with BaP, *trans*-chlordane, and endrin. Pyrene tended

to partition similarly in Lake Michigan water and elutriates even though the mean percent of bound pyrene differed between the partitioning methods examined (Tables 3.3 and 3.4). Overall, BaP showed the most consistant partitioning among the methods.

Contaminant Partitioning after Sediment Manipulation

There were no significant differences in the binding of BaP in porewater and elutriates produced from sediment aged 7 days versus 371 days (t = 0.961, 6 d.f.; Table 3.5), where almost all BaP was bound. Binding in porewater and elutriates produced from *trans*-chlordane and pyrene dosed sediments was variable and did not follow a linear trend with the amount of sediment aging or manipulation. Differences in thepercent of bound contaminant were seen between elutriates and porewaters prepared from the same sediment containing *trans*-chlordane, pyrene, and endrin (*trans*-chlordane, t = 2.364, 11 d.f.; pyrene, t = 4.170, 18 d. f.; endrin, t = 6.180, 9 d.f.; Table 3.5). For all phases and components, the least percent of binding was found in elutriates and porewater produced from endrin dosed sediment. Only about 10% of endrin was bound in elutriates prepared from aged sediment, and stayed relatively constant with sediment manipulation. Binding of endrin in porewater was 2 to 3 times that of elutriate fractions, and changed significantly after sediment manipulation (Table 3.5).

Partitioning over Bioassay Testing Intervals

Because the driving force behind this work was evaluation of bioaccumulation data, the bioavailable fractions of contaminants in porewater and elutriates were followed over the time course of a typical bioassay. The bioavailable fractions in porewater and elutriates stayed relatively constant from 1 to 96 hours in bioassays using midge larvae (Table 3.6). The percent of freely dissolved BaP, *trans*-chlordane, and endrin was significantly different in porewater versus elutriates produced from the same sediment (BaP, t = -4.323, 10 d.f.; *trans*-chlordane, t = -3.023, 10 d.f.; endrin, t = -3.500, 8 d.f.), with elutriates consistently showing a larger bioavailable fraction (Table 3.6). The percent of freely dissolved pyrene was not significantly different in porewater and elutriates (t = -2.123, 10 d.f.), where approximately half of the pyrene was freely dissolved.

TABLE 3.4

MULTIPLE COMPARISON RESULTS FOR DIFFERENCES AMONG MEAN FRACTIONS OF BOUND CONTAMINANTS OBTAINED IN PHASES OF AQUEOUS MEDIA AND METHODOLOGIES FOR THE CONTAMINANTS USED

	Benzo(*a*)pyrene			*trans*-Chlordane		
	XAD-4	Filtration	Centrifug.	XAD-4	Filtration	Centrifug.
Lake Michigan Water	1A	1A	1A	1A	1B	1A
Porewater	ND*	2A	2A	ND	2B	2A
Elutriate	3A	3A	3A	3A	3B	3A

	Pyrene		Endrin	
	Filtration	Centrifug.	Filtration	Centrifug.
Lake Michigan Water	1A	1B	1A	ND
Porewater	2A	2B	2A	2B
Elutriate	1A	1B	3A	3B

Means with same grouping letter for a method and same grouping numeral for an aqueous phase are not significantly different at $P > 0.05$, using Scheffe's multiple range test (*i.e.*, No significant differences were seen among methods that used BaP-dosed Lake Michigan water. However, significant differences were seen for BaP in Lake Michigan water, porewater, and elutriate among all methods).

*Comparison not done

TABLE 3.5

COMPARISON OF CONTAMINANT BINDING IN ELUTRIATES AND POREWATER PRODUCED FROM SEDIMENTS PREVIOUSLY MANIPULATED

Compound	Sediment Age (days)	Days Between Last Mixing and Partitioning Study	% Bound, Porewater	% Bound, Elutriate
Benzo(*a*)pyrene	371	126	100.00 n = 2	100.00 n = 2
Benzo(*a*)pyrene	7	7	99.25 (0.65)* n = 3	95.47 (3.3) n = 3
Pyrene	219	101	100.00 n = 2	100.00 n = 2
Pyrene	298	253	88.23 (2.7) n = 3	34.97 (1.6) n = 3
Pyrene	306**	8	79.28 (0.3) n = 3	33.10 n = 2
Pyrene	60	28	88.82 (2.0) n = 3	47.01 n = 2
trans-Chlordane	371	126	65.90 n = 1	41.70 n = 2
trans-Chlordane	60	28	96.73 (3.1) n = 3	89.52 n = 2
trans-Chlordane	7	7	83.96 n = 2	64.52 (3.4) n = 3
Endrin	298	253	34.91 (4.0) n = 3	10.74 (0.8) n = 3
Endrin	306	8	23.68 (0.9) n = 3	10.46 n = 2

All values were obtained via Sep Pak separation of freely dissolved and bound fractions after centrifugation at 20,000 *g*.

* $\pm$ 1 S. D.

** Sediment was used from previous (298 day old) assay, conducted 8 days previous.

TABLE 3.6

PARTITION COEFFICIENTS AND PERCENT FREELY DISSOLVED CONTAMINANTS IN ELUTRIATES AND POREWATER OVER THE TIME COURSE OF BIOACCUMULATION ASSAYS

Compound	Exposure Period (hours)	Porewater		Elutriate	
		% Freely Dissolved	log K_p	% Freely Dissolved	log K_p
Benzo(*a*)pyrene	1	24	5.25	53	4.90
	6	27	5.18	41	5.11
	24	14	5.52	38	5.17
	48	8	5.79	28	5.36
	72	19	5.38	32	5.28
	96	23	5.28	48	4.99
trans-Chlordane	1	37	4.97	52	5.25
	6	36	5.00	52	5.25
	24	20	5.36	37	5.52
	48	13	5.59	37	5.52
	72	22	5.29	38	5.50
	96	25	5.23	81	4.65
Pyrene	1	53	4.56	64	4.79
	6	45	4.71	55	4.95
	24	54	4.55	50	5.05
	48	46	4.69	47	5.10
	72	48	4.66	58	4.91
	96	36	4.87	54	4.96
Endrin	1	62	4.41	79	3.25
	6	56	4.53	95	2.58
	24	62	4.41	72	3.43
	48	55	4.54	65	3.56
	72	49	4.65	72	3.41

All values were obtained via Sep Pak separation of freely dissolved and bound fractions after filtration through glass fiber filters.

Discussion

From a practical standpoint, centrifugation and filtration methods yielded the most convenient techniques for determining partition coefficients in aqueous samples. Both methods took less time to perform than the XAD resin or dialysis methods. The centrifugation method produced the most consistent partition coefficients for each of the four contaminants tested. Partition coefficients obtained from the filtration method were less consistent, where individual samples differed by up to a factor of 692 for pyrene in dosed Lake Michigan water. The variation in partition coefficients may be due, in part, to differential sorption of the contaminants to the glass fiber filters and glassware used in the technique. Previous studies have used scintillation cocktail to rinse the filtering apparatus, with subsequent LSC (Eadie *et al.* 1990, 1992). These studies showed an average 4.3 to 9.2% of the total contaminant mass sorbed to the filtering apparatus. Such rinsing may have reduced the variability of the K_ps that I obtained and lowered mean partition coefficients. Another factor contributing to the variation in K_p values may be the inability to determine exact TOC concentrations in individual samples. Total organic carbon samples were taken from representative samples of the aqueous fractions studied. The TOC concentration from XAD and filtration/Sep-Pak methods reported in Tables 3.1 and 3.2 was lower than that of the centrifuged supernatant from the centrifugation/Sep-Pak method. The variation in TOC between methodologies was probably due to sediment differences (aliquots of sediment were obtained over a period of several months) or aqueous TOC composition over the course of the study. Sample to sample variation of TOC may have resulted in an overestimation or underestimation of the calculated partition coefficients.

Fewer surfaces were available for contaminant sorption in the centrifugation method, compared to the filtration method. Sorption to the stainless steel centrifuge tubes averaged less than the 40-63% previously reported for fluoranthene and DDE (Schults *et al.* 1992). Centrifuging the samples at 20,000*g* may have lessened the chance for colloids and other microparticulates to be included in the freely dissolved fractions of samples. This may have contributed to the narrower range of K_p values obtained with the centrifugation method.

Partition coefficients obtained from the filtration method compare favorably with previous results of BaP and pyrene in Lake Michigan water (Landrum *et al.* 1985, 1987). In a study that compared filtration/SepPak techniques with dialysis, partition coefficients were significantly higher using dialysis (Landrum *et al.* 1984). Although the partitioning of only one compound (BaP) in elutriate fractions could be

compared using filtration and dialysis techniques in this study, K_p values were comparable for both methods.

The low recovery obtained with dialysis due to sorption onto surfaces may have been reduced by using dialysis membranes composed of a different material. Reliable results have been achieved when Spectra/Por 6 dialysis tubing was used (Carter and Suffet 1982, McCarthy and Jimenez 1985*b*). The dialysis tubing used in the present study was composed of a cellulose ester that may have sorbed the contaminants more readily than the Spectra/Por 6 material made of regenerated natural cellulose. A study designed to compare sorption differences between the two types of dialysis tubing would test this idea.

The fact that partitioning did not significantly change from 1 to 96 h in bioassays indicates that the bioavailability of the contaminants stayed relatively constant over the course of the exposures. Previous studies that examined partitioning between porewater and sediment particles in laboratory-dosed Lake Michigan sediment found partitioning of pyrene and phenanthrene to increase with contact time between 3 and 150 days (Landrum *et al.* 1992 *c*). In those studies, amphipods exposed to aged whole sediment experienced a loss of bioavailable contaminants with sediments aged up to 60 days, reflected in lowered uptake rate coefficients. Such results may depend on the conditions of the experiment (test duration of 96 h in this study was relatively short), the contaminant, and the indicator species used.

Pyrene was the only contaminant that did not partition differently between porewater and elutriates generated from the same sediment. Pyrene was also the least hydrophobic of the contaminants used. The differences in the percentages of freely dissolved contaminants between porewater and elutriate fractions for the three other contaminants suggests that elutriates may not bind these contaminants to as large an extent as porewater. Certainly, the length of contact time between the aqueous phase and sediment particles is shorter with prepared elutriates than with porewater generated from dosed sediment. However, it is possible that more soluble compounds, such as pyrene, equilibrate in aqueous phases more quickly than do less soluble compounds.

Significant differences in TOC composition among the aqueous fractions may have contributed to the differences in partition coefficients obtained for a single contaminant. Although contaminant concentrations in elutriates, porewater, and lakewater were normalized for the quantity of TOC in the samples (equation 3), the quality of TOC contained in dissolved organic material (DOC) most likely differed

among samples. Previous studies showed that partitioning of organics such as BaP and napthalene is proportional to the hydrogen/carbon ratio of DOM in natural waters (Kukkonen 1991). Furthermore, the proportion of hydrophobic acids in DOM significantly correlates with contaminant partitioning (Kukkonen and Oikari 1991). Variation in the proportions of hydrophobic acids, hydrogen/carbon composition, and the amount of microparticulates and colloids were most likely responsible for both the relatively wide range of K_p values obtained among replicates of a single media and the significant differences in K_p values among porewater, elutriate, and lake water obtained for a single contaminant. Of the four methods studied, I obtained the greatest precision from reverse-phase column separation after centrifugation at 20,000*g*. This technique evidently excluded colloids and microparticulates, and was the most successful method for obtaining the most accurate measure of the "freely dissolved" phase of the contaminants in aqueous media.

Partitioning of contaminants in aqueous fractions after sediment manipulation tends to be compound dependent. BaP in aqueous fractions obtained from newly dosed and aged sediment was almost all bound. However, a threefold difference was seen in the amount of binding among elutriates obtained from pyrene-dosed sediment, and binding did not tend to reflect the duration of the contaminant-sediment interaction. The data show that the researcher should be aware of changes in the bioavailability of contaminants in fractions obtained from whole sediment when designing and analyzing the results of toxicokinetic studies.

FEEDING SELECTIVITY AND ASSIMILATION OF PAH AND PCB IN *Diporeia* SPP.

Introduction

Neutral hydrophobic compounds, such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) readily sorb to organic particulate matter and tend to accumulate in sediment. Resistance of these chemicals to biodegradation extends their residence time in the benthic environment. The various routes of contaminant uptake that influence the transfer of sediment-associated contaminants to benthic organisms depend on feeding behavior and characteristics of the sediment and contaminants. Accumulation of sediment-associated contaminants may occur either via the aqueous phase or through ingestion of contaminated particles. Accumulation via the ingestion route depends on feeding rate, assimilation efficiency, and contaminant concentration on the ingested food particles. Some estimates have been made concerning the assimilation of carbon and potassium analogs in aquatic species (Ivlev 1939*a,b*, Welch 1968, Gerking *et al.* 1976, Rasmussen 1984) and assimilation of hydrophobic organics by aquatic invertebrates (Muir *et al.* 1983, Klump *et al.* 1987, Lee *et al.* 1990, Lydy and Landrum 1993). Methods to determine assimilation efficiency have included: (1) direct measurement, using total organic carbon as a tracer (Lee *et al.* 1990, Lydy and Landrum 1993); (2) the dual radiotracer approach that utilizes the ratio of an assimilated to an unassimilated radioisotope (Klump *et al.* 1987, Lydy and Landrum 1993, Klump *et al.* 1991, Lopez and Elmgren 1989); and (3) estimation from the ratio of the rate of excretion of absorbed radiotracer to the total rate of excreted radiotracer (Gerking *et al.* 1976, Muir *et al.* 1983). Assimilation efficiency is defined as accumulation efficiency, or the ratio between the amount of compound absorbed by the gut and the amount ingested (Gerking *et al.* 1976). Each of the methods has produced a relatively wide range of efficiencies among individuals of the same species. Therefore, the processes by which invertebrates assimilate sediment-sorbed contaminants and the factors that influence these processes require additional study to better define the role of ingestion in the bioaccumulation process.

Some selective-feeding benthos consume particles with higher organic carbon concentrations and smaller size than non-selective feeders (McMurthy *et al.* 1983, Adams 1987, Klump *et al.* 1987). In general, these fine materials (< 63 μm) contain the bulk of the sorbed organic contaminants and have been identified as a major